Analysis of the Changes in the Structure and Hydration of the Nucleosome Core Particle at Moderate Ionic Strengths

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ABSTRACT: In order to better understand the conformational changes induced in the nucleosome core particle by changes in the ionic strength of the media in the range from 0.1 to 0.6 M NaCl, we have conducted a very detailed structural analysis, combining circular dichroism, DNase I digestion, and sedimentation equilibrium. The results of such analysis indicate that the secondary structure of both DNA and histones exhibits small (~5%) but noticeable changes as the salt increases within this range. In the case of DNA, the data are consistent with a trend toward a more relaxed secondary structure. The DNase I pattern of digestion is also altered by the salt and suggests a DNA relaxation around the flanking ends. From the hydrodynamic measurements, we also observe a significant change in the virial coefficients of the particle as the salt increases, which in turn are in very good agreement with the theoretically expected values. Furthermore, the preferential hydration parameter is also found to increase with the salt. We believe that the self-dependent conformational change of the nucleosome core particle is the result of the conjunction of all these subtle changes. Yet, from the present data, their exact relationship to the tertiary structure of the whole particle at the different ionic strengths cannot be exactly defined.

Nucleosomes are highly dynamic structural entities, undergoing different conformational transitions both in vitro and in vivo. An example of such conformational versatility is provided by the salt-dependent conformational transition of the nucleosome core particle at moderate ionic strength as indicated by the changes in its frictional parameters (McGhee et al., 1980; Wilhelm & Wilhelm, 1980; Eisenberg & Felsenfeld, 1981; Ausio et al., 1984; Yager & van Holde, 1984). Such conformational change of the nucleosome at ionic strengths in the vicinity of physiological values (0.1–0.6 M NaCl) may provide useful insight into the mechanisms involved in structural transitions of this particle associated with different functional stages of chromatin. Therefore, during the last few years we have concentrated our efforts in trying to ascertain the details of this salt-dependent behavior. In our previous analysis we have shown that the radius of gyration of the particle does not change detectably within the range of ionic strength produced by 0.1–0.6 M NaCl (Greulich et al., 1985). We have also recently shown that release of the histone "tails" in the same salt range (Walker, 1984) cannot account for the change in the frictional parameters since the same percent change in S is observed even if the tails are removed (Ausio et al., 1989). These findings, while ruling out any gross changes either at the DNA or at the histone level, emphasize the subtlety of the mechanisms involved in the salt-dependent conformational transition.

Searching for subtle changes, in the present work we have conducted several experiments in order to check for possible alterations in the secondary structure of both DNA and histones. We also present a careful and detailed analysis of the changes in the virial coefficient and hydration over the same salt concentration range.

Materials and Methods

Nucleosome Core Particles. Nucleosome core particles were prepared as described in Ausio et al. (1989).

Circular Dichroism. Circular dichroism spectra were obtained and analyzed as described elsewhere (Ausio et al., 1989).

DNase I Digestion of Nucleosomes. Nucleosome core particles and core particle size DNA were digested both in 0.1 and 0.6 M NaCl in the presence of 5 mM Tris-HCl, pH 7.5, and 1 mM $MgCl_2$. For the digestion of nucleosomes, ^{32}P 5′-end-labeled particles at 10 μg/mL in either salt were brought to a final concentration of 100 μg/mL by addition of cold nucleosomes under the same buffer conditions. Digestions at 0.1 M NaCl were carried out at 0.8 unit of DNase I/μg of DNA, whereas in those carried out at 0.6 M NaCl the enzyme:substrate ratio was increased up to 8 units/μg of DNA. At these DNase I concentrations, the same amount of total PCA-soluble oligonucleotides could be obtained for the two conditions after a 20-min digestion in 0.1 NaCl and after a 5-min digestion in 0.6 M NaCl. These are the times of digestion for the scans of the samples shown in Figure 4. In other experiments, nucleosomes were digested for different times at different temperatures as specified in the text.

The different conditions of digestion used for both ionic strengths were experimentally established through a preliminary set of pilot digestions. Such differences in enzyme:substrate usage mainly arise from the fact that DNase I works much more slowly (both on naked DNA and on nucleosomes) at 0.6 M NaCl than at 0.1 M NaCl. Such salt-dependent activity seems to be similar to, although less strong than, that exhibited by micrococcal nuclease (Weischet et al., 1979).

The analysis of the DNase I digestion patterns at high salt becomes additionally complicated by the fact that nucleosome core particles show a salt-dependent dissociation behavior (Ausio et al., 1984). Thus, the amount of free DNA present in the sample at the nucleosome concentrations and temperature (0 °C) used for these experiments increases from approximately 5% free DNA at 0.1 M NaCl to ~10% free DNA at 0.6 M NaCl (Ausio et al., 1984). In order to correct for the presence of the free DNA, we designed the following controls: ^{32}P 5′-end-labeled 146-bp nucleosomal DNA (deproteinized) in either 0.1 or 0.6 M NaCl was mixed with cold

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nucleosome core particles containing the same amount of salt (as in the original digestions) in a ratio of 1:10. The final total DNA concentration was 100 μg/mL. DNase I was then added to both samples, and the digestion was performed under the same experimental conditions described above. After the reaction was stopped, these samples were loaded onto the denaturing gel in relative quantities corresponding to 5% or 10% of the total initial loading for the corresponding nucleosome core particles in 0.1 or 0.6 M NaCl. An additional control was prepared by digesting 0.5 μg/mL (5% of the amount used above) ^{32}P end-labeled naked nucleosomal DNA (for 0.1 M NaCl) or 1 μg/mL (10% of the same material) (for 0.6 M NaCl) in the presence of a total final DNA concentration of 100 μg/mL obtained with cold nucleosomes as above. The samples thus obtained were digested under conditions identical with those described earlier. After the digestion was stopped, a volume of sample equal to that used for the original nucleosome samples was loaded onto the denaturing gels. The results from these controls (data not shown) clearly indicated that the contribution to the DNase I pattern by the fraction of free DNA, both in 0.1 and in 0.6 M NaCl, is negligible in both cases, as would be expected from the fact that naked DNA was digested much faster than DNA in the nucleosome under both salt conditions (results not shown).

In all these digestions described above, the reaction was stopped by addition of EDTA at pH 8.0 to the sample to a final concentration of 20 mM on ice. The sample in 0.1 NaCl was then adjusted to 0.6 M NaCl by addition of 4 M NaCl. Then both samples were incubated at 100 °C for 1 min and cooled on ice before the addition of Pronase. Further treatment and preparation of the sample were carried out as described elsewhere (Ausio et al., 1989).

Single-Stranded Denaturing Gel Electrophoresis. Eight percent polyacrylamide denaturing gels containing 7 M urea were prepared in 44.5 mM Tris–borate, 44.5 mM boric acid, and 1 mM EDTA (pH 8.4) buffer and polymerized in the presence of 0.1% ammonium persulfate and 0.05% TEMED at room temperature. Gels were prepared with either a 10:1 or 20:1 acrylamide:bisacrylamide ratio. The lower ratio of cross-linking (10:1) was used to analyze the cutting pattern of DNase I above 60 bp, whereas the 20:1 ratio was routinely used for the analysis of the faster mobility bands. The size of the gels was usually 26 × 48 × 0.1 cm. Prior to the samples being loaded, the gels were warmed by prerunning them until an outer surface temperature of over 40 °C was reached. The samples were then loaded onto the gel, and electrophoresis was continued at ~1500 V so as to maintain the temperature of the gel at around 50 °C. The gels were run until the bromophenol blue was 1 cm from the lower edge (while xylene cyanole was at the middle) of the gel (for a 20:1 cross-linking ratio) or until the xylene cyanole dye was 9 cm from the same edge (for a 10:1 cross-linking ratio).

Analytical Sedimentation. Sedimentation equilibrium experiments were carried out on a Beckman Model E analytical ultracentrifuge. Runs were routinely performed at 20 ± 1 °C at 6800 or 9000 rpm. Six-channel 12-mm cells (Yphantis, 1964) were employed in all the experiments using short solution columns (80-μL sample, 90-μL buffer). The schlieren optical system was used in all the experiments, and the schlieren patterns were analyzed according to Lamm (1929) in order to obtain the apparent z-average molecular weight:

$$M_z^{app} = \frac{2RT}{\omega^2(\partial\rho/\partial c_2)_\mu}\left(\frac{\mathrm{d}\ \ln\ [(1/r)(\mathrm{d}c_2/\mathrm{d}r)]}{\mathrm{d}r^2}\right) \quad (1)$$

The M_z^{app} values thus obtained do not depend on knowledge of the initial starting concentration of the sample. Density contrast variation analysis was performed as described by Eisenberg and Felsenfeld (1981). In this latter case, the nucleosome samples at the different salt and sucrose concentrations had the same concentration (ca. 2.2 mg/mL; A_{260} = 21).

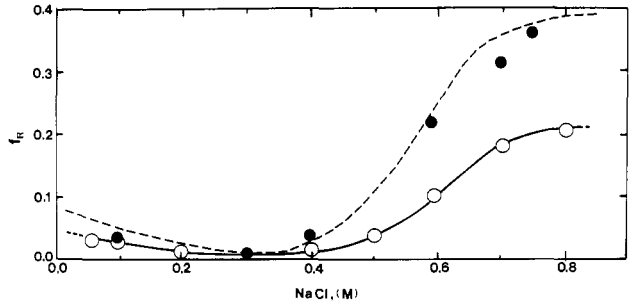

FIGURE 1: Salt dependence of the fraction (f_R) becoming freed from the constraints induced by its interaction with the histone octamer as measured through the increase of the maximum (at 282.5 nm) of the CD spectra. (●) Nucleosome core particles at ~40 μg/mL DNA (i.e., ≃0.8 OD_{260}) and at different salt concentrations were incubated at 22 °C for 14 h before the measurements were performed. (○) Nucleosome core particles at 10 mg/mL DNA (i.e., ≃200 OD_{260}) at different ionic strengths. The dotted line was obtained from previous chicken erythrocyte nucleosome data (Yager, 1984) and from data obtained from nucleosomes prepared from HeLa cells (Ausio & van Holde, 1986). This line, which is used here as a standard, was obtained under sample concentration conditions close to the one used in (●) but without any incubation time. Measurements were performed either in a 1-cm path cell (samples of 0.8 OD_{260}) or in a 50-μm path cell (sample of 200 OD_{260}).

Results

Salt Dependence of the Circular Dichroism of the Nucleosome Core Particle. Upon its interaction with histones, DNA in the nucleosome exhibits an altered circular dichroism spectrum. Particularly in the spectral region of 250–300 nm, which is dominated by the DNA helix conformation, the spectrum seems to be strongly "suppressed" as compared to that exhibited by free DNA.

Two major models have been put forward to account for this phenomenon. One of them, proposed by Fasman (Cowman & Fasman, 1978, 1980; Fasman, 1978), invokes the closely coiled tertiary structure of the DNA in the nucleosome particle. Alternatively, the "suppression" of the spectrum has been assigned to the coexistence of different secondary structures of the DNA in the nucleosome as a consequence of changes in the winding angle of the DNA in its path around the histone octamer. A detailed analysis using subnucleosomal particles containing very short DNA fragments has ruled out the first model (Mencke & Rill, 1982). Therefore, it seems clear that the secondary structure of DNA must change upon its interaction with the histones in the nucleosomes. This idea is also supported by the DNase I digestion pattern of these particles (Lutter, 1979) when compared to B-form DNA lying on a flat surface (Rhodes & Klug, 1980).

There have been several reports indicating changes in the CD spectrum of nucleosomes at moderate salt concentrations (Wilhelm & Wilhelm, 1980; Yager, 1984). A summary of results from our laboratory is shown in Figure 1, which depicts how the ellipticity at the maximum in the nucleosome CD spectrum (at 282.5 nm) increases with increasing ionic strength. It is important to point out here the coincidence of this dependence with that observed for highly hyperaceylated nucleosomes (Ausio & van Holde, 1986) or for trypsinized nucleosome core particles (Ausio et al., 1989). Although these changes might, at first glance, be thought to be symptomatic

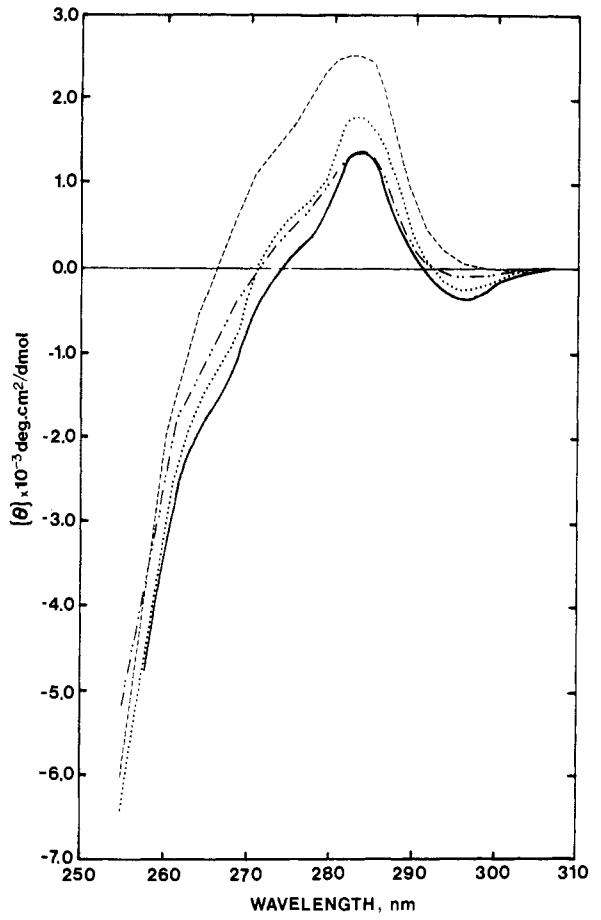

FIGURE 2: Circular dichroism spectra of nucleosome core particles in 0.1 M NaCl in 10 mM Tris-HCl and 0.1 mM EDTA, pH 7.5, buffer (···) or in 0.6 M NaCl in the same buffer (- - -). (—) corresponds to the CD spectrum in 0.1 M NaCl after subtraction of 8% of the CD spectra of naked nucleosome-size DNA in the same salt. (-·-·-) corresponds to the spectrum presented in (- - -) after correction for 18% free DNA at this ionic strength. The values for the amounts of free dissociated DNA under the different salt conditions were obtained from Ausio et al. (1984). In both cases, the spectra for the naked DNA used for the corrections were obtained under experimental conditions identical with those used for the original nucleosome samples. The concentration of the samples was always very close to 0.8 OD_{260}.

of a conformational change, one must be very careful in drawing such conclusions. It must be kept in mind that over this same salt concentration range there occurs a partial dissociation of nucleosomes, yielding increasing amounts of free DNA as the ionic strength increases (Ausio et al., 1984; Yager & van Holde, 1984). When this phenomenon is taken into consideration and the contribution to the CD spectra by the free dissociated DNA is accounted for, the value for the maximum ellipticity at 282.5 nm remains nearly constant within the salt range analyzed here, as shown in Figure 2. Although after such correction the CD spectra are quite similar, some differences remain. For example, the negative peak at 296 nm, which is present at low salt, has significantly decreased at 0.6 M NaCl even after correction for the free DNA. Although the source of this negative ellipticity has never been explained, it may be related to some conformational constraints induced on the DNA by the N-terminal regions of the histones, since this band is abolished by complete trypsinization (Ausio et al., 1989) or extensive acetylation of these histone regions (Ausio & van Holde, 1986). There are also some slight differences in the spectra in the region between 260 and 280 nm. Baase and Johnson (1979) have interpreted the change in ellipticity at 275 nm in terms of changes in the winding angle of the DNA. The differences observed here, however, between 0.1 and 0.6 M NaCl (see Figure 2) may indicate a small change in this parameter. The change in ellipticity we observe at 275 nm, after correction, would only correspond to 4%–5% of the total change in the winding angle per base pair, inferred by Baase and Johnson (1979). The contribution of such a small difference is very difficult to detect at 282.5 nm and, under the low sample concentrations used here, should fall within the experimental error. That this is indeed the case is shown by the salt dependence of the ellipticity at this wavelength when highly concentrated nucleosome samples are used (see Figure 1). In this case, the overall change in ellipticity as a function of salt concentration is smaller, as would be expected from the concentration effects on the salt dissociation behavior of the nucleosome core particles [see Figure 5 in Ausio et al. (1984)]. However, the increase in ellipticity at 282.5 nm expected merely on this basis (~5%–6%) is still somewhat smaller than the experimental value (11%–12%) (see Figure 1). This is in agreement with an ~5% secondary structure change as predicted from the changes in the ellipticity at 275 nm mentioned above.

Below 260 nm, the spectra again change very little with increased salt. We find that the ellipticity at 222 nm of nucleosomal core particles becomes more negative by about 3% as the salt is increased from 0.1 to 0.6 M (data not shown). Although interpretation of such a small change in terms of a change in α-helical content is hazardous for a particle containing DNA as well as proteins, it should be noted that this change is in the same direction and of the same order of magnitude as that reported by Prevelige and Fasman (1987) for free histones. In any event, the data rule out any major change in protein secondary structure.

DNase I Pattern of Digestion of Nucleosome Core Particles in 0.1 and in 0.6 M NaCl. The DNA in the nucleosome core particles when digested with DNase I at low salt exhibits a very characteristic pattern of digestion (Noll, 1974a,b). This pattern arises from the local interactions between the histones and the DNA on its path around the histone core (Lutter, 1979). Thus, the DNase I pattern of digestion of the DNA in the nucleosome is very different from that exhibited by DNA when in solution or when interacting with a flat surface (Rhodes & Klug, 1980). It should then be possible to monitor any changes in the tertiary structure of the DNA in the nucleosomes with the use of this enzyme. With this aim we decided to digest nucleosomes in 0.1 and 0.6 M NaCl with DNase I. However, one of the first problems encountered was the low rate of digestion at 0.6 M NaCl when compared to 0.1 or lower ionic strength. Nevertheless, by increasing the enzyme to substrate ratio, it is possible to partially overcome this problem (see Materials and Methods). Figure 3 shows the DNase I digestion patterns of nucleosome core particles in 0.1 and 0.6 M NaCl at different temperatures. It is obvious from this figure that the patterns of digestion exhibited by the DNA in the nucleosome under the two ionic strengths are different. From inspection of Figure 3, two major differences become immediately apparent. First, there is a significant weakening of the sharpness of the 10-bp repeat in the region below 60 bp from the 3′-end of the DNA. It seems as if the 10-bp repeat has blurred so as to resemble the digestion pattern exhibited by free DNA in solution. Second, the maximal cutting positions of some sites of the 10-bp repeating patterns are visibly shifted. It is important to mention that all the experimental precautions have been taken in order to avoid any possible artifacts arising from the difference in salt concentration between the samples. In each experiment, the

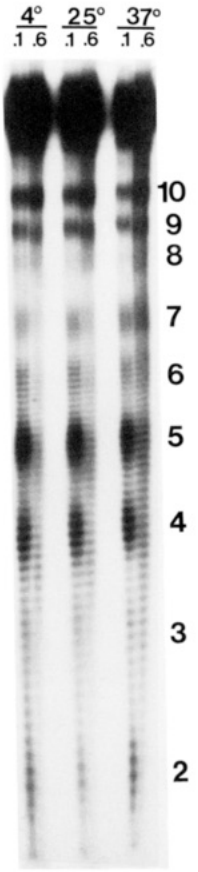

FIGURE 3: DNase I digestion patterns of nucleosomes in 0.1 and in 0.6 M NaCl as a function of the temperature of digestion. Gel electrophoresis was carried out under denaturing conditions at a 20:1 acrylamide to bisacrylamide ratio, as described under Materials and Methods.

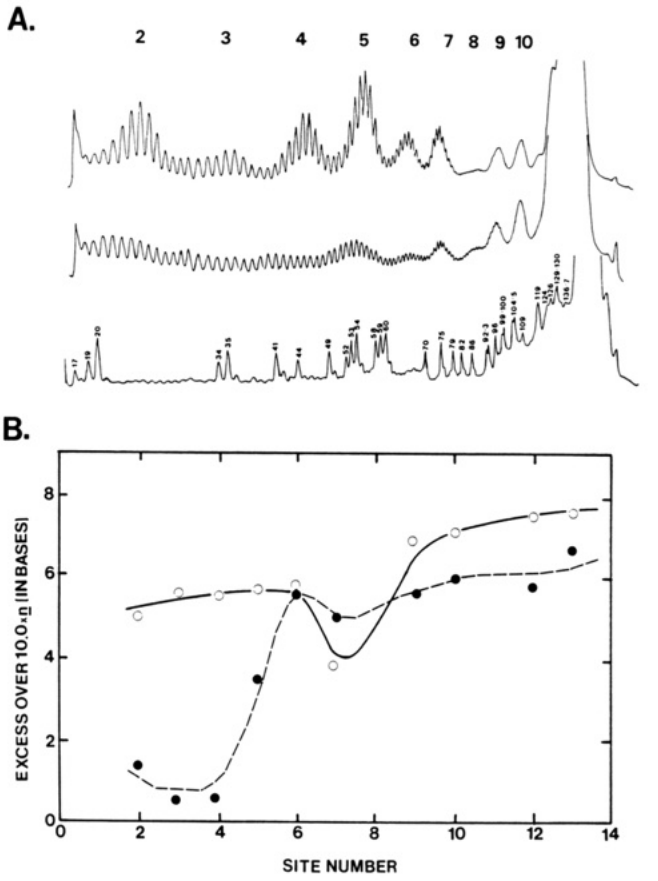

FIGURE 4: (A) Scans of the DNase I digestion patterns of nucleosome core particles at different salt concentrations and at 0 °C in a 10:1 acrylamide to bisacrylamide denaturing gel: upper scan, 0.1 M NaCl; middle scan, 0.6 M NaCl. The marker used (lower scan) corresponds to a DMS-treated 195-bp DNA fragment isolated by *Eco*RI digestion of a pLv 405.10 plasmid containing a DNA insert of the 5S ribosomal gene from *Lytechinus variegatus* (Simpson & Stafford, 1983). (B) Graph of the fragment length distribution for the DNase I digestion patterns in 0.1 (○) and in 0.6 M NaCl (●). The data were obtained from the scans of gels performed at different acrylamide to bisacrylamide ratios. The excess in length over $10.0 \times n$ bases (where n is an integer) has been plotted as a function of band number n (Lutter, 1979).

sample from digestion in 0.1 M salt was adjusted to 0.6 M by addition of concentrated NaCl. After that, the two samples were treated identically and loaded side by side on the gel. We performed more than ten independent experiments and all of them reproduced the same observations mentioned above. Several controls were also taken in order to avoid artifacts arising from the different degrees on DNA dissociation at the different salt concentrations and temperatures used (Ausio et al., 1984). From these controls, the contribution of free DNA to the DNase I pattern was found to be negligible both in 0.1 and in 0.6 M NaCl for the different temperatures used. As a matter of fact, when free 5′-end-labeled nucleosomal DNA (in an amount corresponding to 8% of the labeled DNA in the nucleosome samples) was digested in the presence of a 100-fold excess of cold nucleosomes at 0.1 M NaCl under the same digestion conditions used for end-labeled nucleosomes in this salt and then run side by side in the same gel, the lane corresponding to this amount of free DNA did not show any footprint by the time that the nucleosome counterpart was fully autoradiographed. The same was also true when an amount corresponding to 18% of hot 5′-end-labeled free nucleosomal DNA was digested in the presence of 0.6 M NaCl. [The values for the estimated amount of dissociated DNA at 0.1 and 0.6 M NaCl were taken from Ausio et al. (1984).] The same results were obtained when labeled free nucleosomal DNA in an amount equal to the amount of DNA present in the labeled nucleosome sample was digested separately and then loaded onto the gel in quantities corresponding to 8% or 18% of the starting sample.

Figure 4A shows the scans of one of the gels of 5′-end-labeled nucleosomes digested at 0 °C in the presence of 0.1 and 0.6 M NaCl. These data have been used in order to obtain the data presented in Figure 4B, plotted in the manner described by Lutter (1979). The interpretation of this latter figure is not as simple as in Lutter (1979). It is important to point out here that our digestion conditions are different from those used by Lutter (1979). In our case, we have reduced the amount of divalent ions and also the temperature so as to minimize the dissociation effects. Clearly, as is also evident in Figures 3 and 4, the spacing of positions of maximum cleavage depends on salt concentration. Furthermore, the data indicate significant changes in the pitch of the DNA in 0.6 M NaCl at around 40–60 bp from the 5′-end. Indeed, it has been shown that the site at ~20 bp from the center of the core DNA exhibits an enhanced sensitivity toward micrococcal nuclease when the temperature of the digestion is decreased to 4 °C (Huang & Garrard, 1986; Libertini et al., 1988). DNA seems to adopt a strong kinked configuration around this position as evidenced by the intercalating behavior of methylene blue (Hogan et al., 1987). Furthermore, the altered DNase I regions observed by us agree fairly well with the sites of DNA bending observed by Richmond et al. (1984).

Virial Coefficient and Hydration of the Nucleosome Core Particle at Moderate Ionic Strengths. When X-ray scattering is used to analyze the nucleosome salt-dependent conforma-

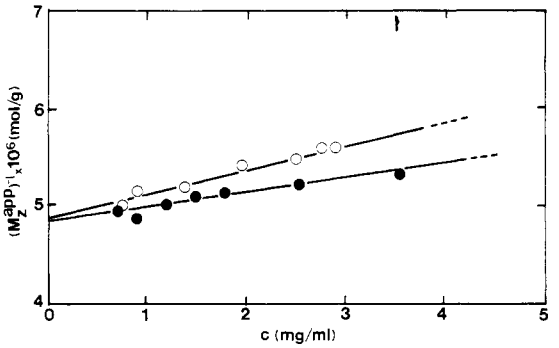

FIGURE 5: Dependence of the reciprocal of the apparent molecular weight (z-average) on concentration for the nucleosome core particles in 0.1 M NaCl (○) or in 0.6 M NaCl (●). The concentration of the nucleosome core particles in the sample was calculated from the absorbance of the sample at 260 nm by using an extinction coefficient for the nucleosome core particle equal to 9.5 cm^2 mg^{-1} (Ausio & van Holde, 1986). Sedimentation equilibrium runs were performed at 6800 rpm at 20 ± 1 °C.

tional transition (Greulich et al., 1985), no change in the radius of gyration of the particle is observed within the salt range 0.1–0.6 M NaCl. Yet, a significant decrease in $I(O)$ (intensity at O angle) is observed when the salt increases from 0.1 to 0.6 M. Since $I(O)$ is proportional to the apparent molecular weight (M^{app}) multiplied by a "contrast factor"

$$I_X(O) \simeq (\partial\rho_{el}/\partial c_2)_\mu^2 M_2^{app} c_2 \quad (2)$$

where X = X-ray diffraction and ρ_{el} = electron density, then a decrease in $I(O)$ must therefore be accompanied by a corresponding decrease in the M_2^{app} (apparent molecular weight) of the particle or in the contrast. At the high particle concentrations required by this technique (≃10 mg/mL), the salt-dependent dissociation of DNA from the nucleosome particles cannot account for the decrease in M_2^{app} since under such concentrations the amount of dissociated DNA even at 0.6 M NaCl and room temperature is estimated to be quite small (≤4%) (Ausio et al., 1984). It is also important to mention here that below 0.8 M NaCl there is no loss of mass due to histone dissociation (Yager & van Holde, 1984).

The fact that the frictional parameters of the particles increase without any change in the radius of gyration at the same time as the zero angle scattering decreases prompted us to undertake a careful hydrodynamic analysis of the virial coefficients of the nucleosome particle at 0.1 and 0.6 M NaCl. The results of such analyses are shown in Figure 5. From this graph it is possible to evaluate both the molecular weight of the particles and their virial coefficients:

$$(M_z^{app})^{-1} = M_z^{-1} + 4Bc_2 \quad (3)$$

where B = colligative second virial coefficient as defined in Roark and Yphantis (1969) and $M_z = M_2$ for homogeneous samples. The molecular weight (z-average) estimated from both sets of data was 205 800, which is in excellent agreement with the value calculated from the composition of the particle: 205 400 for a nucleosome with a 146-bp DNA. The virial coefficients were found to be 6.1×10^{-5} mL mol/g^2 for nucleosomes in 0.1 M NaCl and 3.8×10^{-5} mL mol/g^2 for nucleosomes in 0.6 M NaCl in 10 mM Tris-HCl, pH 7.5, buffer. These experimental values for B compare very well with the estimated values calculated from either equation:

$$B = B_{ex} + B_D \qquad B' = B'_{ex} + B_D \quad (4)$$

where B_{ex} = contribution to the virial coefficient due to the excluded volume and which for a sphere of radius R is

$$B_{ex} = 16\mathcal{N}\pi R^3/3M_2^2 \quad (5)$$

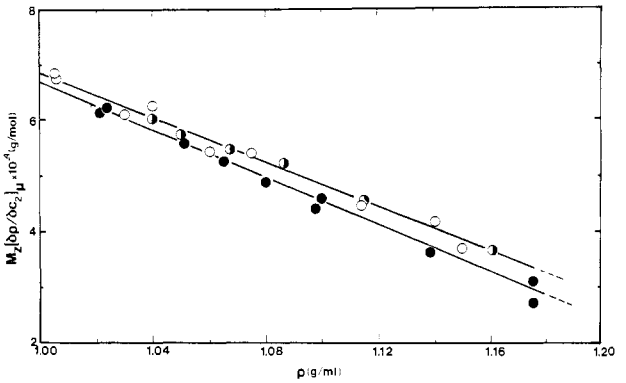

FIGURE 6: Slopes $M_z(\partial\rho/\partial c_2)_\mu$ from sedimentation equilibrium versus medium density (ρ) carried out in sucrose at 0.1 M NaCl (○), 0.3 M NaCl (◑), or 0.6 M NaCl (●), in 10 mM Tris, pH 7.5, buffer. Sedimentation equilibrium runs were performed at 9000 rpm at 20 ± 1 °C for 96 h.

Alternatively, we may express the excluded volume in terms of the partial specific volume $\bar{v}_2$:

$$B'_{ex} = 4\bar{v}_2/M_2 \quad (6)$$

In eq 4, B_D is the Donnan contribution to the virial coefficient and is given by

$$B_D = (z\delta)^2/4M_2C_3 \quad (7)$$

where Z is the total net charge of the particle, C_3 is the molar concentration of the salt in the buffer, and δ is the effective fraction of charge remaining ionized after counterion condensation, which for DNA is equal to 0.24 (Record et al., 1978; Manning, 1978). In these calculations we have used R for the nucleosome core particle = 5.5 nm, $\bar{v}_2$ = 0.667 mL/g (see below), and z^- = 78 (292 negative charges due to the phosphates in the DNA −214 positive charges due to the argiinne and lysine residues of the histones). The values thus estimated were $B = 6.3 \times 10^{-5}$ mL mol/g^2 and $B' = 3.4 \times 10^{-5}$ mL mol/g^2 in 0.1 M NaCl and $B = 4.6 \times 10^{-5}$ mL mol/g^2 and $B' = 1.7 \times 10^{-5}$ mL mol/g^2 in 0.6 M NaCl. Such values, although they should only be taken as approximate *estimates*, agree very well with the experimental ones. The difference observed for the virial coefficients between 0.1 and 0.6 M NaCl, however, cannot explain the drop in $I(O)$ seen by the X-ray scattering, since the change is in the wrong direction. Nucleosomes are *less* nonideal in high salt, and therefore the apparent molecular weight [and $I(O)$] should *increase* with salt at these high concentrations.

Yet another explanation for the change in $I(O)$ could arise from a dramatic change in the hydration of the particle upon increase of the ionic strength of the buffer from 0.1 to 0.6 M NaCl, which would affect the contrast factor. To check for this alternative possibility, we conducted a whole series of density contrast variation experiments in the ultracentrifuge (Eisenberg & Felsenfeld, 1981; Greulich et al., 1985). The results of such analysis are summarized in Figure 6. As is clearly seen in this figure, an increase in the ionic strength from 0.1 to 0.6 M NaCl is accompanied by an increase in the preferential hydration parameter of the nucleosome particle. The preferential hydration parameter ξ_1 was evaluated from

$$(\partial\rho/\partial c_2)_\mu = 1 + \xi_1 - \rho(\bar{v}_2 + \xi_1\bar{v}_1) \quad (8)$$

(Eisenberg, 1976), where ρ = density of the buffer, c_2 = concentration of the nucleosomes, and $\bar{v}_2$ and $\bar{v}_1$ are the partial specific volumes of the particle and water, respectively. Given that M_z = 205 800 and $\bar{v}_1 = 1$, the $\bar{v}_2$ and ξ_1 values obtained

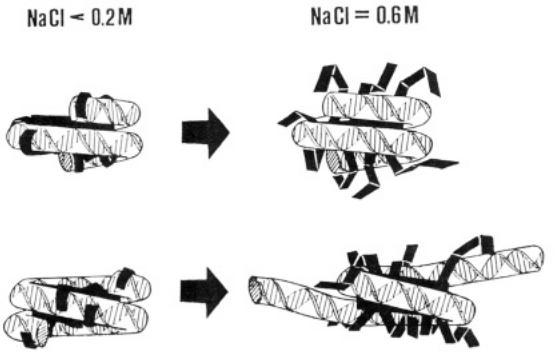

FIGURE 7: Schematic representation of two possible models in order to account for the salt-dependent changes of the frictional parameters of the nucleosome core particles: (top) release of the N-terminal region of the histones; (bottom) partial release of the flanking DNA ends.

from the intercepts and slopes of the lines shown in Figure 6 were $\bar{v}_2 = 0.667$ mL/g and $\xi_1 = 0.301$ g of H_2O/g of nucleosome for nucleosomes in 0.1 and 0.3 M NaCl and $\bar{v}_2 = 0.675$ mL/g and $\xi_1 = 0.361$ g of H_2O/g of nucleosome for nucleosomes in 0.6 M NaCl. From the salt dependence on the preferential hydration parameter, ξ_1, it seems unlikely that the changes in hydration observed here could, only by themselves, account for the changes in $I(O)$ observed with the small-angle X-ray scattering.

Discussion

The two most simple mechanisms to account for the salt-dependent conformational change of the nucleosome core particle are schematically represented in Figure 7. Both models satisfy the experimental observations made by different groups (McGhee et al., 1980; Wilhelm & Wilhelm, 1980; Eisenberg & Felsenfeld, 1981; Ausio et al., 1984; Yager & van Holde, 1984) of an increase in the frictional parameters of the particle as the salt increases in the range of ionic strength below the point at which selective histone dissociation from the DNA begins (around 0.8 M NaCl) (Burton et al., 1978). The first of these models, proposed by Ausio et al. in 1984, was based on the earlier observations made by Cary et al. (1978) and later by Walker (1984) that the N-terminal regions of the histones become mobile and eventually dissociate from the DNA in this salt range. However, it has recently been shown that complete removal of these histone tails does not abolish the salt-dependent conformational transition (Ausio et al., 1989).

The second model (release of DNA ends), which had been proposed earlier (Harrington, 1982), seems at first sight more suitable inasmuch as it would represent an intermediate stage in the DNA dissociation process observed in this range of ionic strength (Ausio et al., 1984; Yager & van Holde, 1984). However, again such model is in serious conflict with the constancy of the radius of gyration. Obviously, other more complicated models might be constructed. Nevertheless, all of them would have to fulfill the seemingly existing paradox of an important change in the frictional parameters of the particle while maintaining constant its radius of gyration. As was mentioned in the introduction, subtle conformational changes may be involved in this structural transition. In this context, we have carefully checked for possible changes in the secondary structure of both histones and DNA. From the circular dichroism section under Results, we find that only a 5% change is observed in both cases. In the case of the spectral region above 250 nm, we have shown that the changes in the spectra as a function of salt are mainly due to the partial DNA dissociation from the particle at different salt concentrations. This is true for the low concentrations of the sample normally used in these kinds of experiments, which therefore rules out the correlation between the changes in the CD spectrum and those observed for the frictional parameters of the particle, as had been proposed earlier (Wilhelm & Wilhelm, 1980). Our interpretation is in very good agreement with the observation made by Walker (1984) using long H1-stripped polynucleosome chains. In that case (where the DNA dissociation is now almost completely abolished), it was found that the CD spectrum did not change when the salt concentration was increased from low salt to 0.6 M NaCl. The interpretation given by Walker to such finding was a decrease in the supercoiling of the DNA as a consequence of the histone tail release (Walker, 1984). This conclusion, although supported by the changes in the sedimentation coefficients of the same kind of polynucleosomes, does not necessarily need to be invoked in order to properly interpret the CD in view of the data presented here. Nevertheless, our DNase I digestion patterns at 0.6 M NaCl seem to corroborate also the relaxation of the supercoiling of the DNA, especially by loosening its histone interaction constraints at the flanking ends of the nucleosome particle, as observed by Walker (1984). As a matter of fact, from the CD results obtained at high concentrations of the sample and also from the changes in $[\theta]_{275}$, a slight but noticeable change in the secondary structure of the DNA (~5%), as mentioned above, seems to take place within this salt range. This change in the DNA seems also to be accompanied by an almost quantitatively identical change in the secondary structure of the histone octamer detected by the changes in the CD spectrum at 222 nm.

At this point it is difficult to relate such changes in the secondary structure of the histones to any major change in the tertiary structure of the histone octamer such as that proposed by Chung and Lewis (1986) when using reconstituted nucleosomes containing fluoresceinated H4. It seems possible that the H4 rotation proposed by these authors might account for all or part of the change in frictional parameters. However, we do not believe that the transition involving H3, postulated by Cantor et al. (1981), can explain our data, since studies with H3–H3 cross-linked histones show no effect on the transition (Ausio et al., 1984).

Any change in tertiary structure must also involve the DNA conformation, as evidenced by the changes in DNase I pattern in 0.6 M NaCl as mentioned above. The loss of the 10-bp periodicity toward the ends of the pattern suggests a loosening or relaxation of the DNA in these flanking regions. On the other hand, an important shift in the sites of the DNase I cleavage is also observed, which seems to have a major effect in the pitch of DNA in the region at 40–60 bp from the 5′-ends. We do not have at present any clear interpretation of this phenomenon.

We have shown that the virial coefficients of the nucleosome particle can be accounted for from the known dimensons and volume of the particle when the contribution of the charge is also taken into consideration. Our predicted values for B and the observed ones are in very good agreement. They are, however, significantly smaller than the value of $B = 2.8 \times 10^{-4}$ mL mol/g^2 previously reported by Greulich et al. (1985) using small-angle X-ray scattering. In the present case, we do see a difference in the virial coefficient between 0.1 and 0.6 M NaCl, which is expected as a consequence of the reduced Donnan effect in high salt. Furthermore, we observe a significant change in the preferential hydration parameter in going from 0.1 to 0.6 M NaCl (see Figure 6). Such a change cannot only affect the $\Delta I(O)/c_2$ but also the frictional pa-

rameters of the particle. Indeed, the values for f/f_0 for the nucleosome core particle in 0.1 M NaCl (f/f_0 = 1.50) or in 0.6 M NaCl (f/f_0 = 1.70) (Ausio et al., 1989) change to f/f_0 = 1.30 and 1.40 at 0.1 and 0.6 M NaCl, respectively, when the ξ_1 values found here are taken into account.

Thus, it seems likely at this point that the salt-dependent conformational transition of the nucleosome core particle arises from the confluence of several subtle alterations in the physical properties of the particle involving the secondary structure of its chemical components (histones and DNA) and also the global extent of hydration. However, the exact relationship of these changes to the tertiary structural changes exhibited by the whole particle, as envisaged in circular dichroism, DNase I digestion, and sedimentation behavior pattern, remains yet to be established.

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