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Structural Changes of Nucleosomes in Low-Salt Concentrations†

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ABSTRACT: We report transient electric dichroism studies of the unfolding of 140 base pair calf thymus nucleosomes in the salt concentration range from 26 mM down to 0.1 mM. A single unfolding transition was found, occurring within the range 0.3–3 mM, with a midpoint at ~1.3 mM. A concentration of 100 μM Mg^{2+} is sufficient to reverse completely the unfolding, yielding the native structure. Nucleosomes cross-linked with dimethyl suberimidate do not undergo unfolding in low-salt solution. The unfolding transition is characterized by an increase in the negative limiting reduced dichroism from 0.29 to 0.48 and an increase in the field-induced, viscosity-limited, rotational orientation time from 0.8 to 1.9 μs. The results imply a model for the low-salt structure consisting of a 178-Å diameter disk, 60 Å in thickness, containing 140 base pair deoxyribonucleic acid (DNA) wound in 0.9 superhelical turn. The low-salt structure orients by a permanent dipole mechanism, with a dipole moment directed along the C_2 symmetry axis of 2600 D, compared to 1200 D for the native 140 base pair nucleosome. At least two to three counterions are released when the nucleosome unfolds. We propose that electrostatic repulsion between adjacent DNA sections in native nucleosomes is primarily responsible for unfolding. This repulsion is relieved in the structure having only 0.9 superhelical turn since the overlap of 0.4–0.75 turn of DNA is lost. We estimate that the free energy of forming the low-salt unfolded structure under physiological conditions may be as small as 7 kcal mol^{-1}, making it an energetically plausible candidate for an enzyme-induced intermediate in functional unfolding of nucleosomes.

The association of conformationally altered nucleosomal particles with transcriptionally active structural genes (Weintraub & Groudine, 1976; Garel & Axel, 1976) and replicating chromatin (Seale, 1978) attracts current interest in the conformational state and transitions of nucleosomal particles. Recently, several studies have been reported on the effect of low salt on chromatin or nucleosomes using electron microscopy (Tsanev & Petrov, 1976; Oudet et al., 1977), hydrodynamics and light scattering (Gordon et al., 1978), and fluorescence (Zama et al., 1977; Dieterich et al., 1977). Most of these studies showed an alteration or transition of structure induced by low salt, but the detailed structures still remain unknown.

Transient electric dichroism with its dual characteristics of rotational relaxation time and reduced dichroism, and a recently developed theory (Crothers et al., 1978), is a sensitive method to study the size, shape, and orientation of DNA molecules free in solution (Hogan et al., 1978), in nucleoprotein complexes (Klevan et al., 1977; Crothers et al., 1978), and in bacteriophages (Kosturko et al., 1979). We report here electric dichroism measurements on the structural transition of nucleosomes induced by low salt. The origin and mechanism of unfolding of nucleosomes in low salt are also discussed.

Materials and Methods

Preparation of Nucleosomal Core Particles. The 140 base pair nucleosomal core particles were prepared by micrococcal nuclease digestion of H1 depleted chromatin as described by Klevan & Crothers (1977) with some minor modifications. The digestion was continued for 10–15 min, and the products were assayed by DNA gel electrophoresis to obtain more homogeneous 140 base pair nucleosomal core particles. After elution from a Bio-Gel A5m column, nucleosomes were run through 5–20% sucrose gradients in an SW27 rotor at 25 krpm for 30 h to remove any small free DNA fragments.

Dichroism Measurement. Dichroism amplitude and relaxation time were measured on a modified T-jump apparatus as described previously (Hogan et al., 1978). Nucleosome stock solutions were made in 10 mM Tris-HCl and 1 mM Na_2EDTA, pH 8 (TE buffer); 100 × TE buffer or 1/100 TE buffer (pH 7.5) was added to the stock solution to obtain the desired salt concentration. Ionic strengths of the solutions were calculated by assuming complete ionization of Tris-HCl and Na_2EDTA at pH 7.5. The rotational correlation time was determined by the viscosity enhancement method as described previously (Klevan et al., 1977). The reduced dichroism did not show any appreciable temperature dependence from 0 to 25 °C. No measurable changes in dichroism amplitude or

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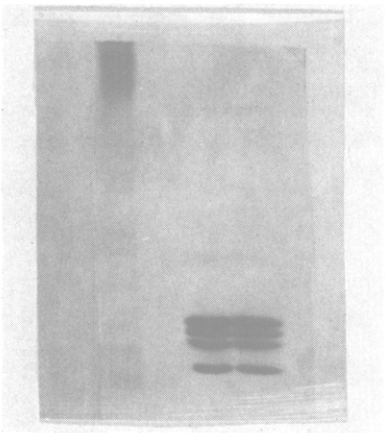

FIGURE 1: Fifteen percent polyacrylamide–NaDodSO$_4$ gel electrophoresis of histones. From left to right: histones from DMS cross-linked nucleosome, from unfolded nucleosome, and from standard compact nucleosome.

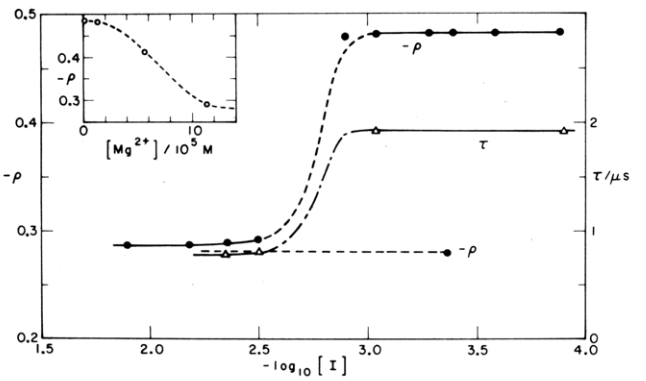

FIGURE 2: Dependence of reduced dichroism extrapolated to infinite field strength (●) and rotational relaxation time (△) on the logarithm of the ionic strength (I). The dashed horizontal curve indicates the constant dichroism found for cross-linked nucleosomes. Insert: effect of added magnesium ions on the dichroism (ρ) of nucleosomes in I = 0.4 mM.

relaxation time could be detected when samples were exposed to repeated application of up to a 35 kV cm^{-1} electric field in low-salt solution.

Analytical Procedure. To assay the association of histones with DNA in nucleosomes in low-salt solution, we dialyzed the nucleosome samples against the appropriate dichroism buffer overnight and then ran them through 5–20% sucrose gradients in an SW40 rotor at 38 krpm for 18 h. The nucleosome peak was collected, lyophilized, and loaded onto histone gels as described by Laemmli (1970).

Nucleosomes were cross-linked with dimethyl suberimidate (DMS) in 10 mM sodium tetraborate buffer, pH 10, and purified on sucrose gradients as described by Stein et al. (1977). Histone gel electrophoresis results for cross-linked nucleosomes are shown in Figure 1. The ionic strength of sample buffers was checked by conductivity measurements to ensure that dialysis was complete.

Results

Nucleosomes show one structural transition between 26 and 0.1 mM ionic strength. Figure 2 shows the limiting reduced dichroism (extrapolated to infinite field; Crothers et al., 1978) and the extrapolated rotational correlation time of nucleosomes as a function of the logarithim of the ionic strength. One transition is observed between 3.5 and 0.65 mM salt for both the reduced dichroism and the correlation time. The same transition also appears in the variation of dipole moment with

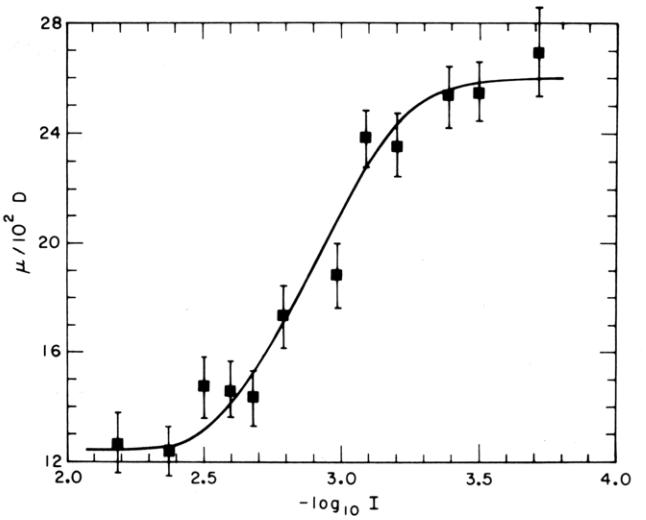

FIGURE 3: Variation with ionic strength (I) of apparent nucleosomal dipole moment (■), expressed in units of 100 D. Dipole moments were determined from the field dependence of the dichroism, as described earlier (Crothers et al., 1978), and the bars indicate computer-calculated standard deviations in the determination of μ from dichroism measured at five different field strengths.

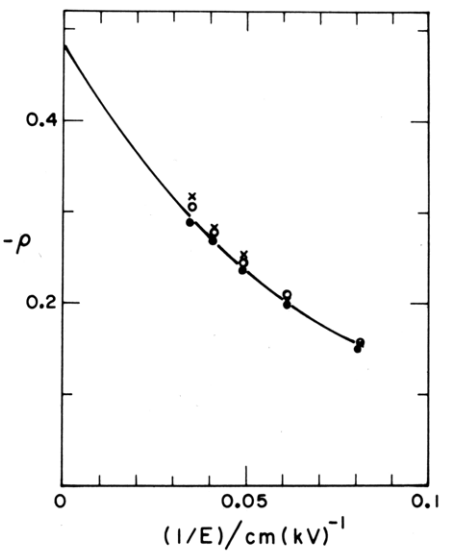

FIGURE 4: Field dependence of the dichroism of nucleosomes in low-salt solution: (●) I = 0.43 mM; (×) I = 0.33 mM; (○) I = 0.22 mM. The solid curve shows the theoretical dependence of ρ on $1/E$ for a dipole moment of 2300 D and a limiting dichroism at infinite field of ρ = −0.48.

salt as shown in Figure 3. The dichroism and the relaxation time of nucleosomes which were cross-linked by DMS in salt concentration between 26 and 3.5 mM retain the same values upon dilution to lower than 0.6 mM salt, as shown in Figure 2. Addition of urea to the low-salt nucleosome sample (ionic strength lower than 0.65 mM) to a final 5 M urea concentration increases both the dichroism and the relaxation time to those values observed in 5 M urea and regular salt buffer (unpublished results).

Low Mg^{2+} ion concentration causes refolding of nucleosomes to the compact state. As shown in the inset to Figure 2, Mg^{2+} at roughly 100 μM concentration is sufficient to reverse the limiting dichroism amplitude to its value for the compact state. The rotational orientation time was also reversed by Mg^{2+} addition to the value found for native nucleosomes (data not shown).

Nucleosomes in low salt possess an apparent permanent dipole moment. As shown in Figure 4, decreasing the ionic

strength of the dichroism buffer from 0.6 to 0.1 mM has no appreciable effect on the measured dichroism at each value of the electric field. This result demonstrates that nucleosomes apparently orient in the electric field by a permanent dipole mechanism in low salt, just as they do in higher salt solution (Crothers et al., 1978). This result also can be shown from the variation of dipole moment with salt (Figure 3). [We take a dipole moment independent of salt concentration as evidence for a permanent moment (Crothers et al., 1978; Klevan et al., 1977).]

The relaxation time implies that nucleosomes increase in volume on unfolding. For an asymmetric particle having a permanent dipole moment, the minimum value of the longest distance across the particle (A_{min}) is $A_{min} = [2kT\tau/\pi\eta]^{1/3}$, in which τ is the rotational orientation time in the field, η is the solution viscosity, and k is the Boltzmann constant (Tanford, 1961; Crothers et al., 1978). The orientation time τ for nucleosomes in low salt is 1.9 μs, obtained by extrapolation of the time constant in dextran solution (Crothers et al., 1978). With no assumption about the shape of the particle, A_{min} for nucleosomes is 146 Å in salt buffer of concentration less than 0.65 mM. For comparison, A_{min} for nucleosomes in regular salt is 110 Å. This indicates that the nucleosome definitely increases in volume in low salt.

The dichroism amplitude implies that nucleosomal DNA unfolds into a fractional circle in solution lower than 0.65 mM salt. We assume that the nucleosome in low-salt conditions retains a C_2 symmetry axis perpendicular to the DNA superhelical axis. A molecule which has a C_2 axis can have a dipole moment only along the C_2 axis. Because of the observed permanent dipole moment and negative reduced dichroism, we conclude that a nucleosome in low salt still orients along its C_2 axis, just as in higher salt solution (Crothers et al., 1978).

The measured limiting dichroism for nucleosomes in low salt is −0.48, whereas it is −0.29 for 140 base pair nucleosomes (taken as 1.4 superhelical turns) in regular salt (Crothers et al., 1978). This together with the implication of nucleosome unfolding from relaxation time data indicates that nucleosomal DNA in low salt must unfold into less than 1.4 superhelical turns. The largest possible negative dichroism for a superhelical DNA with superhelical turns between 1 and 1.4 and orienting perpendicularly to its superhelical axis is −0.375 (Crothers et al., 1978). Therefore, the measured limiting dichroism indicates that nucleosomal DNA must unfold in low salt into a structure containing less than 1 superhelical turn.

The nucleosome in low salt can be modeled as a 168 × 168 × 60 Å swollen disk with 0.9 superhelical turn of DNA. The dichroism amplitude of a superhelical DNA with x fractional turns, oriented perpendicularly to its superhelical axis, can be calculated from eq 1 (Crothers et al., 1978), in which ρ =

$$\rho = (-3/8)(3\langle\cos^2\alpha\rangle - 1)(3\cos^2\beta - 1 - 3B\sin^2\beta) \quad (1)$$

reduced dichroism amplitude, α = angle between DNA base transition moment and double-helical axis, β = superhelical pitch angle (90° for a circle), and $B = \sin 2\delta/[2(\pi - \delta)]$, where $\delta = 2\pi(1 - x)/2$ and x = number of fractional turns.

Assuming that the nucleosome remains disklike in low salt and using $\alpha = 90°$ and $\beta = 85°$ as for nucleosomes in higher salt conditions (Crothers et al., 1978), one finds $x = 0.9$ superhelical turn for a measured dichroism of −0.48. As shown in Figure 5, a 0.9-turn 140 base pair DNA will have an average radius of ~84 Å, assuming that a 3.4-Å base pair spacing is retained at the outer radius of the disk. This result leads us to a disk model of 168-Å diameter (Figure 5) for the low-salt nucleosome. Our methods are not highly sensitive to the thickness of the disk. However, we find that a disk of 168-Å diameter and 60-Å thickness has a longest distance across the center of 178 Å. By use of the equation given by Koenig (1975) for the oblate ellipsoid model and the permanent dipole moment orientation mechanism, the calculated relaxation time τ = 1.95 μs for a 178 × 178 × 60 Å oblate ellipsoid is in good agreement with the observed value of τ = 1.92 μs. We conclude that the nucleosome behaves as a 168 × 168 × 60 Å swollen disk with 0.9 turn of DNA in low-salt solution.

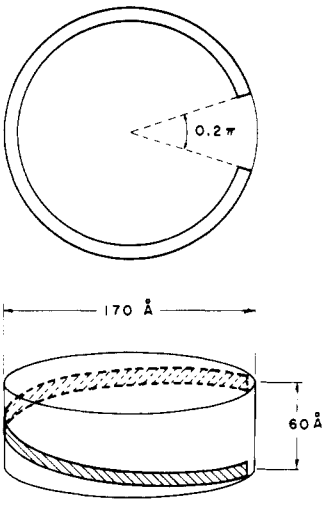

FIGURE 5: Schematic drawing of the proposed model for the structure of nucleosomes unfolded in low salt. Lower: a disk of dimension 170 × 170 × 60 Å; the shaded line represents the path of DNA. Upper: projection of the superhelix viewed along the nucleosomal superhelix axis, indicating 0.9 turn (1.8 π rad) of DNA.

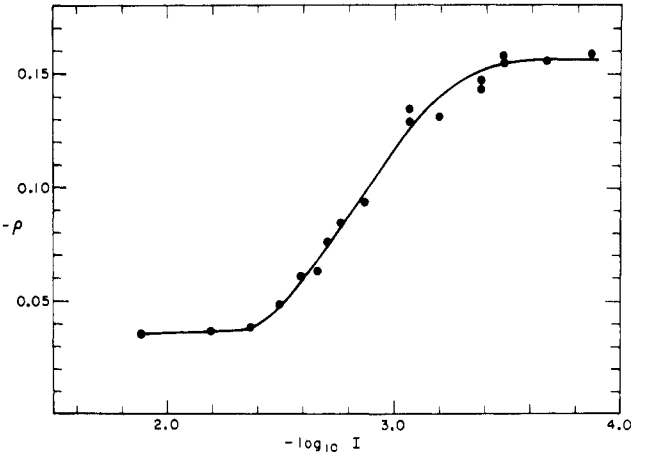

FIGURE 6: Plot of the reduced dichroism (ρ) at 12.3 kV cm^{-1} vs. the logarithm of the ionic strength (I).

At least two to three ions are released when the nucleosome unfolds in low salt. In order to obtain an accurate slope for the unfolding transition, which is needed for calculation of the number of ions that participate, we measured the dichroism ρ at 12.3 kV cm^{-1} for nucleosome solutions in 15 different salt concentrations ranging from 13 mM down to 0.1 mM (Figure 6). The transition curve obtained shows no evidence of intermediate states between the compact and unfolded conformations. Therefore, we make the simplifying assumption that the transition can be approximated by a two-state model (eq 2) in which Nuc and Nuc′ are respectively compact and

$$\mathrm{Nuc}(r) \rightleftharpoons \mathrm{Nuc}'(r') + (r' - r)\mathrm{M} \quad (2)$$

unfolded nucleosomes. The quantities r and r' represent the thermodynamic extent of binding of the salt component M of the solution to Nuc and Nuc′, respectively. Specifically, letting component 1 be water, component 2 be neutral nucleosomes

(with counterions), and component 3 be the added salt (TE buffer), then

$$r = \left(\frac{\partial n_3}{\partial n_2}\right)_{\mu_1,\mu_3,T} \qquad r' = \left(\frac{\partial n_3}{\partial n_2'}\right)_{\mu_1,\mu_3,T} \tag{3}$$

where n is the number of moles of each component and μ is the chemical potential. [Note that as defined by eq 3, r and r' are generally negative. The derivative expresses the number of moles of salt (dn_3) that must be added per mole dn_2 or dn_2' of *neutral* nucleosomes in order to keep constant the chemical potentials of water and salt. Since the charged nucleosome both binds water and releases a fraction of its counterions to the solution, some salt must actually be taken away from the solution in order to keep constant the chemical potential of the salt (μ_3) and water (μ_1) when neutral nucleosomes are added.]

The quantity in eq 2 that can be determined by our experiments is $\Delta r = r' - r$, the moles of salt released in the unfolding transition. Because the nucleosome has a net negative charge, it is natural to think of the salt M as being released because the positive ions are less tightly bound when the nucleosome unfolds. It should be realized, however, that a purely thermodynamic analysis is not capable of making such a distinction between the roles of positive and negative ions.

The equilibrium constant of reaction 2 is expressed by eq 4, in which the observed equilibrium constant K_{obsd} can be

$$K = [\text{Nuc}'][\text{M}]^{\Delta r}/[\text{Nuc}] = K_{obsd}[\text{M}]^{\Delta r} \tag{4}$$

determined from the data in Figure 6. Assuming that the transition is two-state, we can separate the dichroism (ρ_e) of an equilibrium mixture into that of its components (ρ_{Nuc} and $\rho_{Nuc'}$) or

$$\rho_e = \frac{[\text{Nuc}]}{[\text{Nuc}] + [\text{Nuc}']}\rho_{Nuc} + \frac{[\text{Nuc}']}{[\text{Nuc}] + [\text{Nuc}']}\rho_{Nuc'} \tag{5}$$

After some algebraic operations we obtain eq 6, which allows us to calculate the variation of K_{obsd} with salt concentration, using the data in Figure 6.

$$[\text{Nuc}]/[\text{Nuc}'] = K_{obsd} = (\rho_e - \rho_{Nuc})/(\rho_{Nuc'} - \rho_e) \tag{6}$$

Taking the logarithm and differentiating eq 4 with respect to ln [M], noting that K is independent of [M], yields eq 7,

$$\partial \ln K_{obsd}/\partial \ln [\text{M}] = -\Delta r \tag{7}$$

an equation that has been derived and discussed in detail in a number of earlier papers (Wyman, 1964; Zimm & Rice, 1960; Crothers, 1964, 1971; Record et al., 1976).

Figure 7 shows the data for log K_{obsd} plotted against log [M]. The slope is −2.5, indicating that Δr is 2.5. This quantity has the following physical meaning: for each nucleosome that undergoes transition from compact to unfolded conformation, 2.5 molecules of the salt component (TE buffer) must be removed from the solution in order to keep constant the chemical potential of the salt component.

The result that $\Delta r = 2.5$ can be interpreted to mean that *at least* two to three counterions are released from compact nucleosomes when they unfold. There are two reasons why 2.5 is the lower limit on the number of ions released. (1) If the effect is due primarily to release of positive ions on unfolding, more than 2.5 positive ions (up to 2 times as many) would have to be released to require the removal of 2.5 positive *and* negative ions from the solution in order to keep constant the salt chemical potential. (2) If the transition is not strictly all-or-none, and we do not have compelling evidence that it is, then the presence of intermediate states would broaden the transition. This correction could also lead to a larger number than 2.5 ions released.

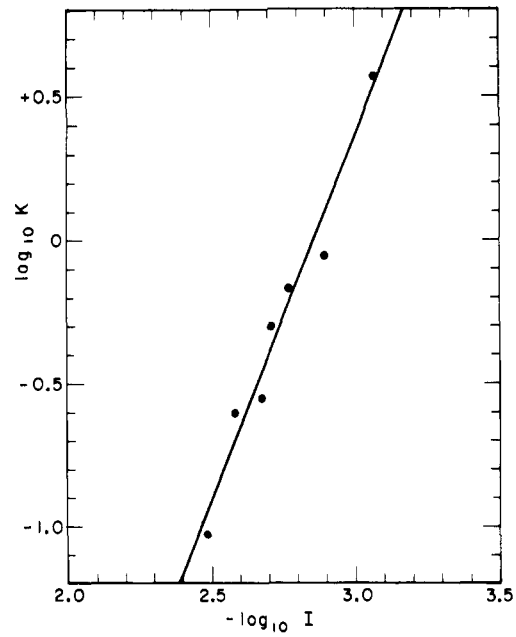

FIGURE 7: Estimation of the number of ions (Δr) involved in the transition using the plot of log K_{obsd} vs. log I. The slope of −2.5 yields $\Delta r = 2.5$.

The theory of Record et al. (1976) for analysis of salt effects on nucleic acid binding equilibria goes beyond the strictly thermodynamic analysis presented here, using the molecular theory of Manning (1969, 1972) for linear polyelectrolytes to interpret Δr in terms of the number of salt linkages formed in a protein–nucleic acid complex. However, application of that theory does not seem appropriate here, because of the globular shape of nucleosomes and our lack of detailed knowledge concerning their local charge density.

Discussion

We found only one structural transition between 26 and 0.1 mM ionic strength. Dilution of the nucleosome core particle solution from 13 mM to lower than 1.2 mM ionic strength causes the particles to unfold from a 110 × 110 × 55 Å compact disk to 168 × 168 × 60 Å ring-shaped particles, while nucleosomal DNA relaxes from 1.4 superhelical turns (Crothers et al., 1978) to ~0.9 turn. This structural transition as monitored by the reduced dichroism and relaxation time occurs in a relatively broad range of ionic strength from 3.5 to 0.65 mM with the midpoint around 1.3 mM. Recently, Gordon et al. (1978), using hydrodynamic and light-scattering methods, reported the observation of two structural transitions of nucleosomes around 1 mM Na^+ and 7 mM Na^+, but we could not resolve the single transition observed between 13 and 0.65 mM into two transitions and could not find any other transition, even when the salt concentration was reduced to 0.1 mM. However, the addition of urea, final concentration 5 M, to a nucleosome sample in 0.5 mM solution causes both the dichroism and relaxation time to increase from −0.48 and 1.9 μs to −0.7 and 6 μs, the same values as for nucleosomes in 5 M urea and higher salt solution. This implies that the low-salt nucleosome is still in a compact structure compared with the high urea nucleosome and can be unfolded further. This excludes the possibility of a similar DNA structure for nucleosomes in low salt (lower than 1 mM) and high urea as proposed by Gordon et al. (1978). We should point out that our measured frictional parameter differs from that of Gordon et al. (1978) since the relaxation time obtained from electric dichroism is proportional to the rotational diffusion coefficient,

whereas light scattering gives the translational diffusion coefficient. Recently, Dieterich et al. (1977), using fluorescent labeled nucleosomes, also observed only one transition in low salt.

Other Evidence for General Unfolding of Nucleosomes. Substantial changes are observed in the rotational and translational diffusion constants of nucleosomes in low-salt solution. This alteration can be prevented by cross-linking the histone proteins together with DMS. These observations argue strongly for a substantial change in nucleosome size and shape, accompanied by an alteration of protein tertiary structure or protein–protein contacts (quaternary structure). Since most of the DMS cross-linking occurs in the N-terminal part of the histones (Wyns et al., 1978), it is probable that the N-terminal histone region is involved in the low-salt unfolding process. In addition, it is likely that the histone C-terminal regions open up in low salt, as shown by the fluorescence change displayed by nucleosomes labeled at the cysteine residue of H3 (Zama et al., 1977; Dieterich et al., 1977), which is located in the C-terminal portion of the molecule. All of these results imply a widespread conformational change of nucleosomes in low salt.

Indirect support for our model of a 178-Å diameter disk for the low-salt form is provided by the electron microscopy observations of Seligy & Poon (1978), who found 174-Å diameter ring-shaped particles in partially heat-denatured nucleosomes. Of course, it is not necessary that thermal and low-salt unfolding proceed through the same intermediates, but nevertheless their results and ours seem to imply similar unfolded structures.

Electrostatic Effects in the Unfolding of Nucleosomes. Decreasing the ionic strength of the solution reduces the ability of the solvent to screen the Coulombic interactions between the charged groups on nucleosomes. One expects this effect to be most pronounced for the long-range repulsive interactions that result from the net negative charge of the nucleosome. A 140 base pair nucleosome contains 280 negatively charged phosphate groups, with 33 additional negative charges from the histone proteins, and only 118 positive charges contributed by the lysine, argine, and histidine residues of the eight histone proteins (Fasman et al., 1977). It is clear that decreasing the concentration of screening counterions will cause the net electrostatic free energy of the particle to increase. A structural transition will occur if there is another structure whose electrostatic free energy is lower, presumably because the alternative structure has larger size and a smaller net negative charge density.

Our model for the initial and final states of the unfolding transition provides a plausible specific explanation for the reduction in electrostatic free energy. The native 140 base pair nucleosome contains either 1.75 (Finch et al., 1977) or 1.4 (Crothers et al., 1978) superhelical turns of DNA. Therefore, two 40–60 base pair segments of DNA lie adjacent to each other on the circumference of the disk, with the edges of the DNA double helixes separated by only ~5–10 Å. Removal of the screening counterions increases the repulsion between these overlapping portions of DNA. At a critical salt concentration (~1.3 mM) the disk expands to 0.9 turn of DNA, removing the overlap of DNA segments. This motion requires an energetically unfavorable distortion of the histone proteins but results in a favorable reduction of electrostatic free energy. When the salt concentration is low enough, the latter effect dominates and unfolding results. Mg^{2+}, which is expected to chelate between the overlapping DNA segments, should strongly stabilize the native structure, as is observed.

Local repulsion among positive charges may contribute to nucleosome instability. In spite of the net negative charge density of the nucleosome, it is possible that there are local regions of net positive charge density whose mutual repulsion contributes to the unfolding free-energy reduction in low salt. For example, the positively charged N- and C-terminal portions of histones H2A and H2B remain mobile in nucleosomes (Cary et al., 1978). Removal of screening anions from these sites could increase the electrostatic free energy, resulting in an unfolding roughly analogous to the process of dissociation of the histone octameric core complex, which is stable in 2 M NaCl (Thomas & Kornberg, 1975) but not in low salt solution. Cross-linking of nucleosomes, which occurs primarily in N-terminal regions (Wyns et al., 1978), could act to prevent unfolding by holding the repelling N termini together. Of course, it is also possible that the primary effect of cross-linking is to prevent the tertiary and/or quaternary protein structural changes which are required for unfolding.

Biological Implications. Rapid unfolding and refolding of nucleosomes is one way to accommodate the presence of nucleosomes in transcriptionally active structural genes and replicating chromatin. In such models it is implied that the enzymes have better access to DNA when the nucleosome unfolds. RNA polymerase is able to transcribe 140 base pair nucleosomes, producing primarily 140 base pair transcripts (Bustin, 1978). The recent observation of the low transcriptional efficiency of DMS cross-linked nucleosomes (H.-M. Wu, unpublished experiments) suggests the necessity for unfolding before nucleosome-bound DNA can be an efficient template for RNA polymerase. Whether such functional unfolding might be related to the low-salt unfolding process is unknown. Integration of eq 7 up to higher salt concentrations, with $\Delta r \geq 2.5$, reveals that the free-energy change for forming the low-salt unfolded state may be as small as 7 kcal mol^{-1} under physiological conditions. It is not unreasonable that an unfavorable free energy of this magntidue could be compensated by the favorable interaction of polymerase or other proteins with DNA. In addition, other factors such as histone modification by acetylation may act to facilitate unfolding. We conclude that the low-salt unfolded nucleosomal structure is an energetically plausible candidate for a functional intermediate, but it is also possible that functional unfolding, if it occurs, involves some as yet unknown structure.

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Deoxyribonucleic Acid Excision Repair in Chromatin after Ultraviolet Irradiation of Human Fibroblasts in Culture†

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ABSTRACT: We have exposed confluent normal human fibroblasts to ultraviolet (UV) fluences of 5, 14, or 40 J/m^2 and monitored the specific activity of post-UV repair synthesis in chromatin with [^{3}H]thymidine pulses. We have shown that under conditions where no semiconservative deoxyribonucleic acid (DNA) synthesis is detectable, the specific activity of repair label in micrococcal nuclease resistant (core particle) DNA is about one-fifth that in bulk DNA at all three UV fluences. On the other hand, the distribution of thymine-containing pyrimidine dimers in bulk and nuclease-resistant regions measured either immediately after irradiation or at later times showed no significant differences; preferential labeling of linker (nuclease-sensitive) DNA during repair synthesis is thus apparently not due to a predominance of UV-induced photoproducts in linker relative to core particle DNA in the nucleosome. Pulse and pulse–chase experiments at 14 or 40 J/m^2 with normal human or repair-deficient xeroderma pigmentosum (XP) cells showed that at most 30% of repair label in all these cells shifts from nuclease-sensitive (linker) DNA to nuclease-resistant (core particle) DNA.

Recent work on the structural organization of histones and DNA into repeating monomeric units (nucleosomes) raises interesting questions concerning both the susceptibility of the cellular genome to physical and chemical damage and the accessibility of the genome to enzymes and other possible factors required for the repair of such damage. With respect to the former issue, DNA damage produced by a variety of chemical agents, including trimethylpsoralen plus near-UV light, benzo[*a*]pyrene, dimethylnitrosamine, *N*-acetoxy-2-(acetylamino)fluorene, *N*-hydroxy-2-(acetylamino)fluorene, and bleomycin, has been shown to occur selectively in nuclease-sensitive (linker) regions of nucleosomes (Cooper et al., 1975; Ramanathan et al., 1976a,b; Metzger et al., 1976, 1977; Jahn & Litman, 1977; Cech & Pardue, 1977; Kuo & Hsu, 1978). This distribution of damage is consistent with observations in related studies where DNA repair of certain forms of chemical damage occurs preferentially in linker regions (Ramanathan et al., 1976b; Bodell, 1977; Tlsty & Lieberman, 1978).

It has also been reported (Cleaver, 1977; Smerdon et al., 1978; Smerdon & Lieberman, 1978) that following UV irradiation of human cells, DNA repair as measured by repair synthesis occurs preferentially in linker regions of nucleosomes. However, it has not been established whether or not UV photoproducts such as pyrimidine dimers are similarly preferentially distributed in linker regions of chromatin in intact irradiated cells. The ultimate fate of UV-induced repair synthesis label detected initially in linker regions is also unclear. One group (Cleaver, 1977; J. E. Cleaver and H. Weinbraub, personal communication) has found that repair synthesis label

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