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Conformations of the Core Nucleosome: Effects of Ionic Strength and High Mobility Group Protein 14 and 17 Binding on the Fluorescence Emission and Polarization of Dansylated Methionine-84 of Histone H4†

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ABSTRACT: Chicken histone H4 labeled at Met-84 with the fluor *N*-[(acetylamino)ethyl]-8-naphthylamine-1-sulfonic acid has been incorporated into a nucleosome which has physical characteristics virtually identical with those of native core nucleosomes. The fluorescence emission and polarization properties of the labeled nucleosome were measured as a function of ionic strength and the binding of high mobility group (HMG) proteins 14 and 17. Also, the accessibility of the fluor to the quenching agent acrylamide was determined. It was found that the fluorescence emission changes in the range 0.1–1000 mM NaCl are rather small and indicate that no major unfolding of the octamer structure occurs around Met-84 on H4 at least. Five or perhaps six discrete states were found in that ionic strength range. Each has a different accessibility to the quenching agent. The range of accessibilities varied from 9×10^{-7} to 32×10^{-7} mol^{-1} s^{-1} for 0.1–1000 mM NaCl, respectively. Polarization measurements showed that there was little change in the rotational relaxation lifetime of the fluor at ionic strengths less than 50 mM NaCl. Above this value, the rotational relaxation lifetimes decreased from 107 to 25 ns at 600 mM NaCl, indicating a moderately increased rotational freedom for the fluor. It is suggested that the histone octamer changes its degree of compaction in the range 0.1–600 mM NaCl but that no major protein unfolding occurs. The binding of HMG 14/17 at low ionic strength (10 mM) is noncooperative and appears to increase the compaction of the histone octamer, while at physiological ionic strengths (100 mM) the binding is cooperative and the octamer becomes less compact as reflected by properties of the fluor bound to Met-84 on H4.

The functions of nucleosomes in chromatin, aside from the compaction of DNA, are still indeterminate. While the chromatin of all eukaryotes examined to date contains this ubiquitous subunit, there is much evidence for heterogeneity in nucleosome composition and structure. Nucleosomal DNA from transcriptionally competent chromatin is hypersensitive to digestion by DNase I and other nucleases (Weisbrod, 1982a,b). Histone posttranslational modifications and DNA base methylation further add to the complexity. These differences suggest that nucleosomes might be able to adopt different conformations during transcription. Whether these alternate states are characterized by unwinding of the DNA supercoil from the histone octamer, by unfolding of the histone octamer, or by some combination of both is uncertain.

Many investigators have addressed this question by examining the conformation of core nucleosomes in solutions of different ionic strength and when proteins such as H1 and HMG 14/17 are bound (McGhee & Felsenfeld, 1980; Wilhelm & Wilhelm, 1980; Burch & Martinson, 1981; Dieterich & Cantor, 1981; Daban & Cantor, 1982; Yau et al., 1983; Yager & van Holde, 1984; Ausio et al., 1984). In spite of intensive study, there is still disagreement as to whether or not the nucleosomal histone octamer experiences major unfolding at low (<1 mM) or high (>300 mM) ionic strengths. For example, Uberbacher et al. (1983) found from neutron scattering that only small conformational changes occur in the histone octamer when nucleosomes are transferred to a low ionic strength buffer. In contrast, Cantor and co-workers report that the histone core in a nucleosome becomes flexible at low ionic strength on the basis of the emission of fluors attached to Cys-110 of histone H3 (Dieterich et al., 1977, 1979). For higher ionic strengths in the range 10–600 mM, Ausio et al. (1984), on the basis of sedimentation measurements and cross-linking, found no large structural changes in the nucleosome. On the other hand, Dieterich et al. (1977, 1979) and Daban & Cantor (1982) report that major unfolding occurs by 600 mM NaCl as reflected in changes in the spectral properties of a fluor bound at the same H3 site mentioned above.

Since it has been reported that nucleosomes containing H3 modified at Cys-110 are measurably less stable than unmod-

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ified H3 (Lewis & Chiu, 1980; Wingender et al., 1981; Ausio et al., 1984), it may be that the structural changes observed in H3 Cys-modified nucleosomes are a consequence of this lability rather than being due to an intrinsic property of the nucleosome. Not all sites in the histone octamer are destabilized by chemical modification. Nucleosomes which contain histone H4 modified at Met-84 with a dansyl group, we find, do not suffer from this instability. Kleinschmidt & Martinson (1984) have recently reported that the iodination of H4 tyrosine-72 has no effect on histone octamer stability. They also report that iodination of H2B tyrosines-37, -40, and -42 destabilizes the octamer while iodination of tyrosines-83 and -121 stabilizes this complex.

Here we report the results of a detailed study of nucleosomes containing Met-84 H4 labeled with *N*-[(acetylamino)-ethyl]-8-naphthylamine-1-sulfonic acid (8-AEDANS).[1] Only moderate changes in the fluorescence characteristics of the attached fluor were found to occur in response to changes in ionic strength and the binding of proteins HMG 14/17. From this, we conclude that the histone octamer does not experience major unfolding at either high or low ionic strength. Nevertheless, there is evidence for the existence of discrete conformations, and these correlate reasonably well with the observations of others obtained from techniques which monitor the overall rather than the local structure of the nucleosome (Uberbacher et al., 1983; Yager & van Holde, 1984; Ausio et al., 1984).

Our results are consistent with a histone octamer structure which is always folded but which nonetheless undergoes internal structural changes in response to variations in ionic strength and the binding of HMG proteins. Taken together with the results of earlier studies, we propose that these internal changes result in a relaxation of the binding of the supercoiled DNA to the histone octamer.

Materials and Methods

Histones, HMG Proteins, Core Nucleosomes, and DNA. Electrophoretically homogeneous samples of histones H3 and H4, and an equimolar mixture of histones H2A and H2B, were isolated from histone H1/H5 depleted chicken erythrocyte acid-extracted histones by gel filtration on Bio-Gel P10 (Van der Westhuyzen et al., 1974). Electrophoretically homogeneous HMG proteins 14 and 17 were obtained by preparative electrophoresis from crude fractions isolated by the method of Rabbani et al. (1978). The concentrations of aqueous solutions of these proteins were determined spectroscopically by using published extinction coefficients (D'Anna & Isenberg, 1974; Javaherian & Amini, 1977). The purity of all proteins was determined on 15% acid–urea or 15% SDS–polyacrylamide slab gels (Panyim & Chalkley, 1969; Thomas & Kornberg, 1975). Chicken core nucleosomes were prepared by a modification of the method of Lutter (1978) and core-length DNA by a hydroxylapatite protocol (Simon & Felsenfeld, 1979). This yielded homogeneous 146 bp DNA when sized against an *Hae*III digest of pBR322 DNA.

Fluorescent Labeling of Histone H4. Histone H4 (2 mg) dissolved in 100 μL of distilled water was added to 2 mg of either [5-^{3}H]- or [8-^{3}H]IAEDANS (specific activity 10 mCi/mmol) dissolved in 100 μL of dimethylformamide. The pH was adjusted to 3.5 with glacial acetic acid and the reaction allowed to proceed in the dark for 48 h at 37 °C. The labeled histone was precipitated by the addition of 6 volumes of acetone. Unreacted label was removed by repeatedly washing the precipitate with dimethylformamide–0.5% HCl until a nonfluorescent supernatant was obtained. Following a final acetone wash, the pellet was vacuum-dried and redissolved in 50 mM cacodylate (pH 7). The extent of labeling was determined spectrophotometrically and by scintillation counting. Tritiated 5- and 8-IAEDANS were prepared by a scaled-down version of the method of Hudson & Weber (1973).

Nucleosome Reconstitution and Characterization. Equimolar quantities of H2A, H2B, reduced H3, and labeled H4 (1.2 mg of total protein) were dissolved in 200 μL of 6 M Gdn·HCl, 50 mM Tris, 10 mM EDTA, and 5 mM dithiothreitol (pH 8) which also contained 1 mg of chicken core-length DNA. The mixture was diluted with 1.5 mL of 2.5 M NaCl, 20 mM Tris, and 10 mM EDTA (pH 8) and then dialyzed against 0.6 M NaCl, 10 mM Tris, and 0.7 mM EDTA, pH 8, for 45 min with shaking. After a 45-min incubation at 37 °C, the dialysis medium was changed to 10 mM Tris and 0.7 mM EDTA (pH 8). This reconstitution regime is a modified version of that described by Stein (1979). After 3 h or more in the final dialysis buffer, the reconstitution mixture was clarified by centrifugation and then fractionated on a 5–20% sucrose gradient containing 10 mM Tris and 10 mM EDTA (pH 8). Appropriate fractions containing properly reconstituted nucleosomes were pooled and dialyzed into either 0.1 mM Tris (pH 8) or 10 mM Tris (pH 8). The reconstituted nucleosomes were assayed for homogeneity on 5% Tris–cacodylate–EDTA polyacrylamide gels of the type described by Maniatis et al. (1975) except that borate was replaced by cacodylate and the buffer concentration was reduced to 10 mM. Histone integrity, stoichiometry, the relative DNA content, and the precise amount of labeled H4 incorporated were determined. Thermal denaturation and circular dichroism measurements were performed as described elsewhere (Lee et al., 1982). DNase I digestion experiments of ^{32}P end-labeled native and reconstituted core particles were carried out according to Lutter (1978).

Fluorescence Measurements. Corrected fluorescence emission spectra were obtained with an Aminco SPF 500 corrected spectra spectrofluorometer. All solutions were made from the purest chemicals available. Strict temperature control was maintained, and thermal equilibration was allowed between measurements. Titrations were carried out many times and involved the sequential addition of aliquots of a filtered stock solution to the nucleosome solution. The excitation was at 350 nm, the emission was monitored at 480 nm, and the nucleosome concentrations were in the range (0.5–1.0) × 10^{-7} M, unless otherwise specified. The temperature was 22 °C.

For anisotropy measurements, a polarization attachment for the Aminco SPF 500 spectrofluorometer was used. The two polarizers were motor-driven, and a built-in achromatic scrambler eliminated the need to determine the correction factor for the emission monochromator grating. The anisotropy, A, is given by $A = (I_{vv} - I_{vh})/(I_{vv} + 2I_{vh})$. The fluorescence lifetimes of 8-AEDANS in reconstituted core nucleosomes were measured by using a time-correlated single photon counter as described elsewhere (Ware, 1971; Egan, 1984).

Results

Fidelity of Nucleosome Reconstitution with Labeled H4. Histone H4 specifically labeled at Met-84 with dansyl derivatives (extent of labeling 60–80%) by a sulfonium salt is

[1] Abbreviations: *x*-IAEDANS, *N*-[[(iodoacetyl)amino]ethyl]-*x*-naphthylamine-1-sulfonic acid where *x* = 5 or 8; Tris, tris(hydroxymethyl)aminomethane; EDTA, ethylenediaminetetraacetic acid; 8-AEDANS, *N*-[(acetylamino)ethyl]-8-naphthylamine-1-sulfonic acid; SDS, sodium dodecyl sulfate; bp, base pair(s); Gdn·HCl, guanidine hydrochloride; HMG, high mobility group.

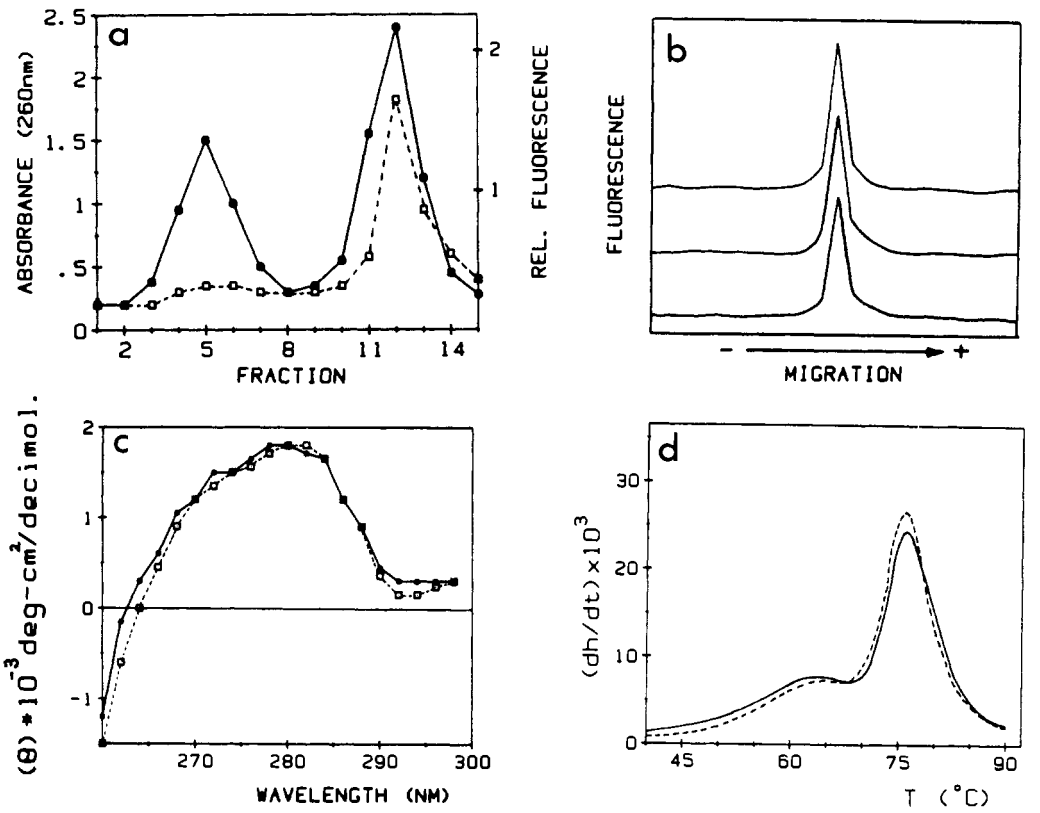

FIGURE 1: Purification and characterization of AEDANS-labeled nucleosomes. (a) Sucrose gradient sedimentation profile of labeled nucleosomes. Free DNA and reconstituted nucleosomes sediment at fractions 5 and 12, respectively [(●) absorbance at 260 nm; (□) fluorescence emission monitored at 480 nm]. (b) Electrophoretic mobility of labeled nucleosomes; 2.5 μg of native (top), control-reconstituted (middle), and AEDANS-labeled nucleosomes (bottom) was electrophoresed on 5% particle gels, stained with ethidium bromide, and scanned for ethidium fluorescence. (c) Circular dichroic spectra of native (●) and AEDANS-labeled nucleosomes (□). The DNA concentration was 75 μg/mL. (d) Derivative thermal denaturation profiles of native (—) and AEDANS-labeled nucleosomes (---) in 0.25 mM EDTA (pH 8).

readily incorporated into a nucleosome during reconstitution as we have reported previously (Lewis, 1979). Reassociation experiments using native core particles in the presence of varying amounts of modified H4 show that the final dialysis supernatant contains nucleosomes with the amount predicted for random exchange. Thus, the presence of label at Met-84 has no detectable effect on whether the labeled histone is incorporated into the nucleosome. Figure 1a shows the separation of labeled core particles on a 5–20% sucrose gradient. The *s* value of the labeled nucleosome is indistinguishable from that of native core nucleosomes (11 S). The first peak on the left of Figure 1a contains free DNA, and some associated histones, but most (>90%) of the dansyl fluorescence comigrates with the core particles in the second peak. Also, the mobility of the labeled nucleosome on 5% particle gels is identical with that of native core nucleosomes as well as control reconstituted core particles containing unmodified H4 (Figure 1b). To further establish that the reconstituted core particles are structurally similar to native particles, we have measured the circular dichroic spectra of these particles between 260 and 300 nm, a region sensitive to DNA conformation. Again, we see no difference between reconstituted and native particles (Figure 1c). In addition, the DNase I digestion patterns of H4-labeled AEDANS nucleosomes, labeled at their 5′ ends with [γ-^{32}P]ATP, were indistinguishable from those of native core particles (data not shown).

Previously, we have demonstrated that the derivative thermal denaturation profile can be an extremely sensitive indicator of perturbed nucleosomal structure (Lewis & Chiu, 1980). Thermal denaturation measurements were made on nucleosomes containing as much as 1.2 labels per octamer (Figure 1d). In no instance could we detect any differences in the melting profiles between control and labeled nucleosomes. This is certainly not true for H3-labeled nucleosomes as we have shown earlier (Lewis & Chiu, 1980).

Fluorescence Emission. The fluorescent chromophore used for this study was the 8-isomer of AEDANS. It was chosen because it exhibits larger changes in its quantum yield when moved from a nonpolar to a polar environment than the 5-isomer (Hudson & Weber, 1973). However, the 5-isomer was also examined so that we could compare our results to those of Cantor and co-workers for labeled histone H3 (Dieterich et al., 1979). The results described here are for the 8-isomer. Essentially the same results were obtained for the 5-isomer, but the magnitude of the changes was typically 60–70% of that for the 8-isomer.

Typical corrected fluorescence spectra for labeled H4 and a nucleosome containing 8-AEDANS-labeled H4 are shown in Figure 2. The emission and excitation maxima for the labeled nucleosomes are 475 and 355 nm, respectively. On the other hand, the emission maximum of the labeled free histone occurs at 487 nm. The measured fluorescence lifetime of 8-AEDANS nucleosomes was 22.3 ns in 10 mM NaCl. These data indicate that the Met-84 site within a nucleosome is experiencing a hydrophobic environment intermediate in polarity to ethanol and dimethylformamide (Hudson & Weber, 1973). The quantum yield of these H4-labeled core particles in 10 mM salt was 0.70 relative to quinine sulfate in 0.05 M H_2SO_4, where quinine sulfate has a quantum yield of 0.70 (Scott et al., 1970). These values taken together suggest that the Met-84 locus on H4 probably experiences a more nonpolar environment than the H3 sulfhydryl site since labels at that site have a quantum yield of 0.61 under similar conditions (Dieterich et al., 1979).

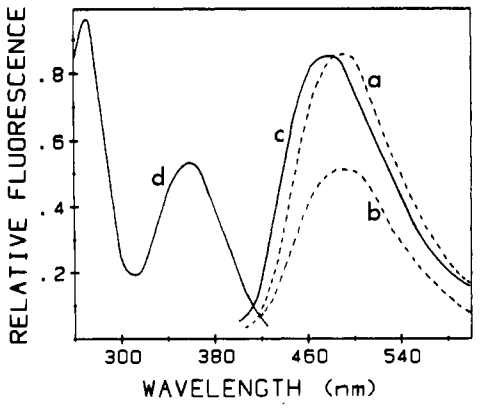

FIGURE 2: Corrected fluorescence spectra of AEDANS-labeled histone H4 and AEDANS-labeled nucleosomes. Curves a and b are H4–AEDANS emission spectra in 10 mM Tris, pH 7.4, and in 100 mM acrylamide and 10 mM Tris, pH 7.4, respectively. Curves c and d are emission and excitation spectra of AEDANS-labeled nucleosomes, respectively. For curve d, the emission was monitored at 480 nm. The free histone H4 concentration was 10 μg/mL. All excitations were made at 350 nm.

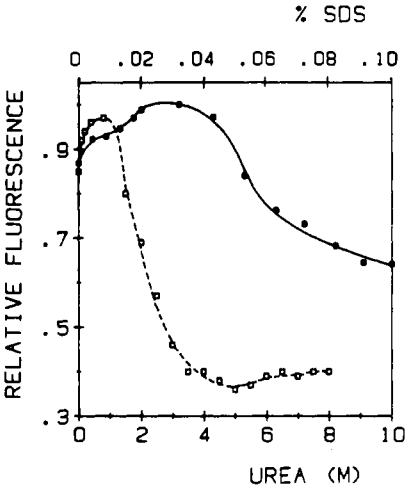

FIGURE 3: Effect of denaturants on the fluorescence emission of AEDANS-labeled nucleosomes. Titration of labeled nucleosomes with SDS (●) and urea (□) is shown. Nucleosomes were initially in 10 mM Tris, pH 7.4. In the range from 4 to 8 M urea, weighed amounts of solid urea were used to change its concentration.

Nucleosome Denaturation. Figure 3 shows the quantum yield changes that occur when the nucleosomes are unfolded by solutions of SDS and urea. With increasing concentrations of SDS, there is an initial increase in the fluorescence emission as the solvent becomes less polar. This is followed by a broad but cooperative transition centered around 0.06% SDS in which the fluorescence emission decreases. As the histones dissociate from the DNA and then from each other, there is about a 40% decrease in the emission intensity. Urea-induced unfolding, in which the histones do not dissociate from the DNA (Olins et al., 1977), has its midpoint at 2 M urea. This results in a maximum 60% drop in fluorescence intensity. The latter environment is more polar than 0.1% SDS, and thus, the emission of the chromophore is quenched more efficiently (Hudson & Weber, 1973). Olins et al. (1977) have monitored the cooperative destabilization of the histone octamer between 0 and 10 M urea. Their data suggest that 50% of the α-helix is denatured at 5 M urea, with no significant changes occurring up to 3 M urea. Our results reveal a cooperative exposure of the Met-84 locus in H4 to the solvent at 2 M urea where no detectable secondary structure changes in the histone octamer have yet occurred. Thus, the environment change around

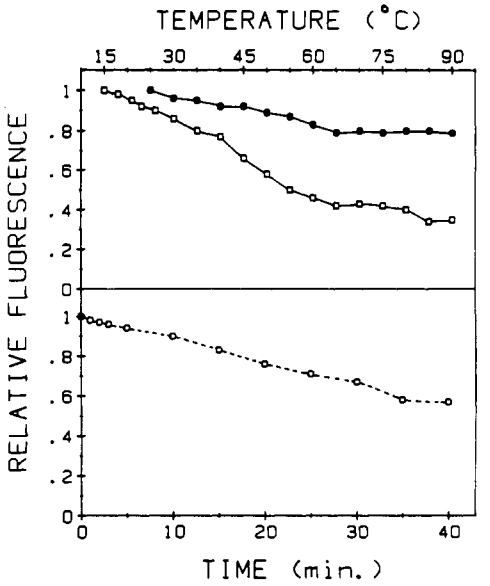

FIGURE 4: Effect of temperature and trypsin digestion on the fluorescence emission of labeled nucleosomes. Thermal denaturation (upper panel) was carried out in the absence (□) and presence (●) of 0.5% SDS. The buffer contained 10 mM Tris and 0.7 mM EDTA (pH 8), and the nucleosome concentration was 20 μg/mL of DNA. During trypsin digestion [lower panel (○)], the buffer was 10 mM Tris (pH 8), and the temperature was maintained at 25 °C. The enzyme concentration was 3 μg/mL, and the nucleosome concentration was 70 μg/mL of DNA. Excitation at 350 nm, emission at 480 nm.

Met-84 in H4 precedes the loss of α-helix in the histones when urea is used as a denaturant. Theoretical and spectroscopic studies on histone H4 in free solution have shown that the α-helix is located at residues 55–67 and possibly at residues 80–92. However, experimental evidence for the precise location of the C-terminal helical region is uncertain. Met-84 is probably located at the N-terminal end of this helix (Crane-Robinson et al., 1977), if it exists at all.

Thermal denaturation of Met-84-labeled nucleosomes results in the gradual exposure of the fluor without any abrupt transitions (Figure 4, upper panel). In the range from 15 to 40 °C, the drop in the fluorescence emission is probably due to collisional quenching with the solvent, since labeled H4 behaves similarly in free solution at low ionic strengths (data not shown). For comparison, we have also unfolded the nucleosomes in SDS prior to thermal denaturation. In this case, the emission decreases monotonically with increasing temperature as would be expected for collisional quenching. Thus, below 40 °C, no major temperature-induced changes occur in the vicinity of Met-84 in the labeled nucleosome. Above 40 °C, we observe a gradual transition centered around 52 °C during which the fluor becomes relatively exposed. Thus, the Met-84 locus of histone H4 is sensitive to temperature-induced unfolding, and the change monitored by the fluor precedes the main melting transition which occurs at 78 °C when thermal denaturation is monitored by UV absorption in 10 mM salt (Weischet et al., 1978). This correlates well with the lower concentration of urea (2 M) needed to reduce the fluorescence emission by 50% as compared to the concentration of urea (5 M) required to half unfold the histone secondary structure as shown in Figure 3. A DNA-specific transition has been reported for the thermal denaturation of the core nucleosome at 50 °C in 1 mM salt, but this transition occurs at a much higher temperature in 10 mM salt (Weischet et al., 1978). Thus, the possibility that the emission decrease is accompanied by DNA structural changes seems unlikely but cannot be excluded.

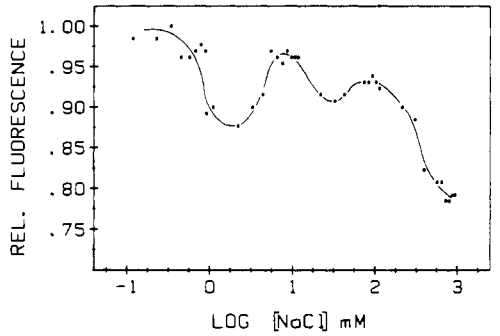

FIGURE 5: Effect of ionic strength on the fluorescence emission of AEDANS-labeled nucleosomes. Nucleosomes were initially in 0.1 mM Tris (pH 7.4). The ionic strength was varied by adding small aliquots of concentrated NaCl stock solutions. The emission was monitored at 480 nm. The excitation was at 350 nm.

Limited trypsin digestion (Figure 4, lower panel) of core nucleosomes results in the cleavage of the N-terminal portions of the core histones without substantially altering the structural properties of nucleosomes (Lilley & Tatchell, 1979; Whitlock & Stein, 1978). Relatively high ratios of trypsin to nucleosome (3 μg of trypsin/70 μg of core histones) were needed to obtain appreciable changes in emission intensity in the Met-labeled nucleosome. After 40 min of trypsin digestion, the histones were extensively degraded (data not shown), although the decrease in fluorescence was only half the limit value obtained had Pronase been used instead. The very high levels of trypsin needed to cause changes in the fluorescence spectrum, taken together with the blue shift in the emission maximum upon incorporation of the labeled histone into a nucleosome, suggest that Met-84 on H4 is not situated on the exterior but is likely buried in the interior of the histone octamer.

Emission Changes with Increasing Ionic Strength. Nucleosomes have been reported to undergo a number of salt-induced conformational changes in solutions of low (Gordon et al., 1978; Dieterich et al., 1979) and moderate ionic strength (Wilhelm & Wilhelm, 1980; Dieterich et al., 1979). An ionic strength titration of the fluorescence emission of the 8-AEDANS Met-H4-labeled nucleosome is shown in Figure 5. In the range from 0.1 mM to 1 M NaCl, four or perhaps five distinct transitions can be seen. Two of these occur at 0.7 and 5 mM NaCl. In this range, Dieterich et al. (1979) observe a single transition at 1.2 mM NaCl from the fluorescence emission of dansyl-modified H3 at Cys-110. Their transition, which involves a much larger change in emission intensity than we observe for our label at Met-84 on H4, was interpreted as being due to the opening up of the histone octamer when the ionic strength was decreased. Dieterich & Cantor (1981), in a subsequent study, report that this transition exhibited complex unfolding kinetics. We note that none of our labeled nucleosome samples exhibited any detectable time-dependent transitions with half-lives of greater than 30 s, our minimum measurement interval.

Beyond 10 mM NaCl but before 100 mM NaCl, we observed one or two small transitions followed by a final major change centered around 250 mM NaCl. Dieterich et al. (1979) found two transitions for their Cys-110 H3-labeled nucleosome, one in the range 0.1–0.35 M NaCl and the other in the range 0.35–0.6 M NaCl. Both transitions were interpreted as being due to the unfolding of the histone octamer and involved much larger changes in emission intensity (>60%) than we have observed (<25%) for the Met-84 H4 AEDANS-labeled nucleosome. One major difference between our results and theirs is that we find the maximum emission intensity to occur at the lowest ionic strength examined, 0.1 mM NaCl. This suggests that the fluor at Met-84 is buried at low ionic strengths in the nucleosome and this is inconsistent with a structure open to the aqueous environment.

Table I: Acrylamide Quenching Rate Constants (k_q) for 8-AEDANS Met-84 H4-Labeled Nucleosomes as a Function of Ionic Strength[a]

[NaCl] (mM)	k_q ($\times 10^{-7}$ mol^{-1} s^{-1})	[NaCl] (mM)	k_q ($\times 10^{-7}$ mol^{-1} s^{-1})
0.1	9	100	11
1.0	18	200	15
10	10	600	24
50	15	1000	32

[a] k_q values were determined by linear regression analysis of the Stern–Volmer plots shown in Figure 5.

Acrylamide Quenching. The salt-induced conformational transitions of a reconstituted core nucleosome containing AEDANS attached at Met-84 H4 as revealed by its emission changes, while quite reproducible, are nevertheless relatively small. To determine whether the emission differences represent real changes in accessibility to solvent and solute molecules or merely reflect changes in the polarity of the dye environment, a series of titrations of the labeled nucleosome were made with acrylamide which is an efficient, nonionic fluorescence quenching agent (Dieterich et al., 1979). Curve b in Figure 2 shows the quenching that occurs when Met-84 AEDANS-labeled H4 is exposed to 100 mM acrylamide.

Addition of 100 mM acrylamide to AEDANS-labeled nucleosomes in 1 mM NaCl results in a 40% reduction of the emission intensity while in 10 mM NaCl the same concentration of acrylamide gives only a 20% reduction. Thus, fluorescence quenching by acrylamide acts as a useful measure of the exposure of the dye to dissolved solutes. Collisional quenching obeys the Stern–Volmer equation

$$F_0/F_c = 1 + k_q t_0 c \quad (1)$$

where F_0 and F_c are the fluorescence emission intensities in the absence and presence of quencher, k_q is the quenching rate constant, t_0 is the fluorescence lifetime of the dye in the absence of quencher, and c is the molar concentration of the quencher. Additional quenching by NaCl can be corrected for by

$$F(\text{cor}) = (F_0/F_c - 1)[F_0(10\text{ mM Tris})/F_0([\text{NaCl}])] \quad (2)$$

where F_0(10 mM Tris) and F_0([NaCl]) are the acrylamide free emission intensities of the labeled nucleosome in 10 mM Tris and at various salt concentrations, respectively. A plot of F(cor) against acrylamide concentration should give a straight line with a slope k_q.

Acrylamide titrations of the AEDANS-labeled nucleosome in the range 0.1–1000 mM NaCl are shown in Figure 6a,b. Each of the ionic strengths corresponds to a local emission maximum or minimum shown in Figure 5. Rate constants for acrylamide quenching at various salt concentrations are given in Table I. The correspondence between the quenching rate constants and the fluorescence emission intensities shown in Figure 5 suggests that the AEDANS at Met-84 on H4 can monitor not only local changes in polarity but also more general accessibility changes. A salt titration of fluorescence emission intensity at 100 mM acrylamide is shown in Figure 6c. We note that the accessibility of the dansyl group at Met-84 on H4 in 10 mM NaCl to acrylamide is very similar to that for the same fluor at the Cys-110 site in H3 used in the studies of Dieterich et al. (1979). However, these authors reported that the label is 4 times more accessible to quencher in 0.1 mM NaCl and 11 times more accessible in 0.6 M NaCl than in 10 mM NaCl. In contrast, we find little difference in accessibility to acrylamide in 0.1 or 10 mM NaCl. How-

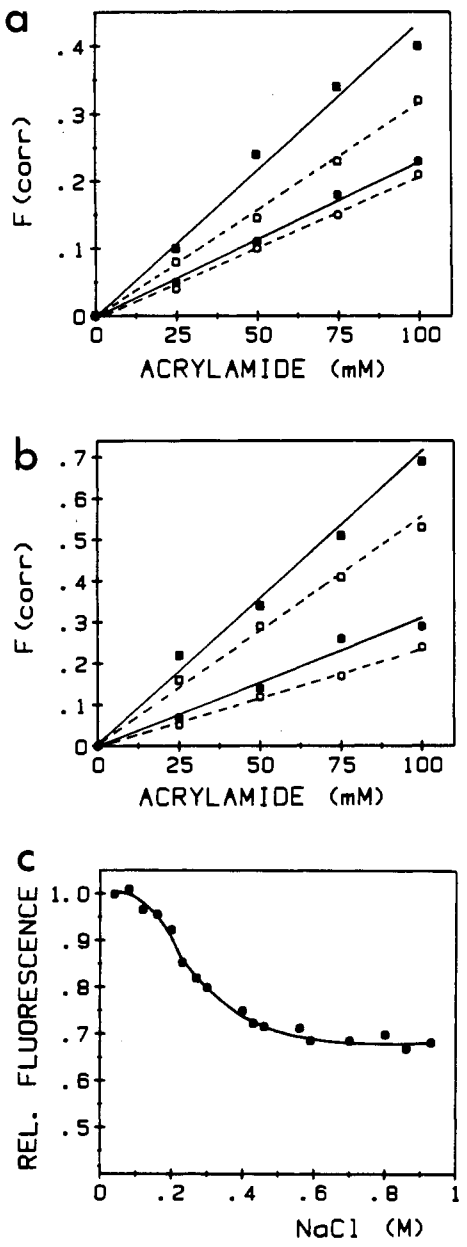

FIGURE 6: Acrylamide quenching of the fluorescence of AEDANS-labeled nucleosomes. (a) Stern–Volmer plots for labeled nucleosomes in 0.1 (○), 1.0 (■), 10 (●), and 50 mM NaCl (□). (b) Stern–Volmer plots for labeled nucleosomes in 0.1 (○), 0.2 (●), 0.6 (□), and 1.0 M NaCl (■). All samples in (a) and (b) contained 0.1 mM Tris (pH 7.4). (c) NaCl titration of labeled nucleosomes in the presence of 100 mM acrylamide (●). Nucleosomes were initially in 10 mM Tris (pH 7.4). Excitation at 350 nm, emission at 480 nm.

ever, at 1 mM NaCl, there is a 2-fold increase in the quenching rate. Our results indicate that the histone octamer, at least around Met-84 in H4, is maximally folded in 0.1 or 10 mM NaCl and becomes less folded in NaCl concentrations around 1, 50, and greater than 100 mM NaCl. However, even by 0.6 M NaCl, we find only a 2–3-fold increase in the quenching rate relative to the 10 mM NaCl value, indicating only partial exposure of the fluor to solvent.

Fluorescence Polarization. Information about the mobility of a bound fluor, as well as the shape of the nucleosome, can be obtained from the analysis of fluorescence polarization data by the Perrin equation (eq 3) which relates the observed an-

$$1/A = (1/A_0)(1 + \tau RT/\eta V) = (1/A_0)(1 + \tau/\tau_R) \quad (3)$$

isotropy (A), the limiting anisotropy in a rigid medium (A_0), the lifetime of the excited state (τ), the viscosity of the medium

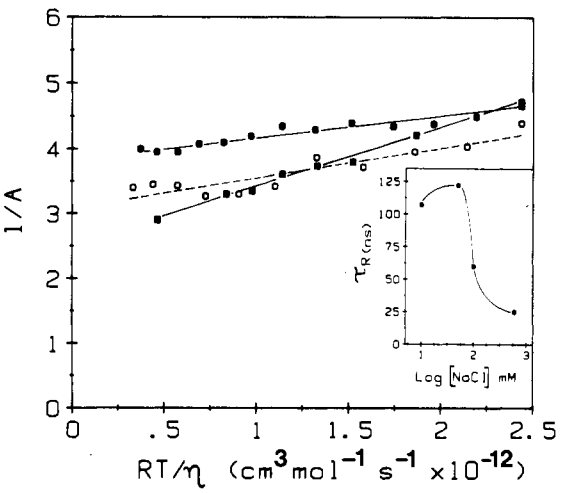

FIGURE 7: Fluorescence polarization of AEDANS-labeled nucleosomes. Perrin plots for labeled nucleosomes in 10 (●), 100 (○), and 600 mM NaCl (■). A is the anisotropy, R is the gas constant, T is the absolute temperature, and η is the viscosity of the medium. All samples also contained 1.0 mM Tris (pH 7.4). The polarization data for the labeled nucleosomes in 50 mM NaCl were similar to those in 10 mM NaCl and have been omitted for the sake of clarity. The inset shows the rotational relaxation times, τ_R, calculated from the slopes of the Perrin plots as a function of the ionic strength. Excitation at 350 nm, emission at 480 nm.

Table II: Fluorescence Polarization Derived Rotational Relaxation Lifetimes (τ_R) for 8-AEDANS Met-84 H4-Labeled Nucleosomes as a Function of Ionic Strength[a]

[NaCl] (mM)	$1/A_0$	V^b	τ_R	τ_R/τ_{R_0}	a/b	dimensions (Å)
10	3.85	2.63	107	1.29	2.0	120 × 120 × 60
50	3.83	2.99	122	1.47	2.6	135 × 135 × 52
100	3.09	1.46	60			
600	2.52	0.61	25			

[a] A_0 is the limiting anisotropy in a rigid medium, V is the molar volume, and a and b are the lengths of the major and minor axes of the oblate ellipsoid use to approximate the nucleosome shape. τ_{R_0} is the relaxation time corresponding to a spherical nucleosome. The dimensions were calculated according to the graphical method of Weber (1953) by modeling the nucleosome as an oblate ellipsoid. [b] Units are $cm^3/mol \times 10^{-5}$.

(η), and the rotational relaxation time of the bound fluorescent dye (τ_R) (Cantor & Schimmel, 1980). τ_R represents the time required for the average value of cos θ to change from unity at $t = 0$ to $1/e$ at $t = \tau_R$ where θ is the angle of depolarization. R is the gas constant and V the effective molar volume of the kinetic unit monitored by the dye. The degree of depolarization of incident linearly polarized light by the fluor depends on the rigidity and dimensions of the macromolecular complex or a portion of the complex to which it is attached. Perrin plots of $1/A$ vs. RT/η are linear with a slope of τ/A_0V and an intercept of $1/A_0$. Once V has been obtained from the polarization data, the rotational relaxation time τ_R of a sphere with that volume can be calculated from

$$\tau_R = \eta V/RT \quad (4)$$

Perrin plots for the 8-AEDANS Met-H4-labeled nucleosomes at various ionic strengths are shown in Figure 7 as are the τ_R values calculated from eq 4. Since the rotational relaxation time for a sphere having the volume of a nucleosome is calculated as 86 ns (Dieterich et al., 1979) and the observed value for an H4-labeled nucleosome is 107 ns at 10 mM NaCl, the labeled nucleosome is clearly asymmetric. A graphical method by Weber (1953) allows the calculation of the axial ratio of an equivalent ellipsoid from the measured τ_R and a

calculated τ_R^{sphere} value. Assuming an oblate ellipsoid, the dimensions can be calculated from the axial ratio and molar volume. The results of such an analysis of our data are given in Table II.

Raising the ionic strength from 10 to 50 mM monovalent ion results in an increase in τ_R and the volume of the equivalent oblate ellipsoid. One interpretation of this result is that the histone octamer has become less compact. This possibility is further supported by the concomitant fluorescence emission decrease and accessibility increase shown in Figures 5 and 6 for this ionic strength increase.

Above 50 mM NaCl, the rotational relaxation times of AEDANS in the H4-labeled nucleosome decrease to 60 ns at 0.1 M NaCl and 25 ns at 0.6 M NaCl. This indicates considerable segmental motion around the fluor but much less than the 5-ns value expected for a fluor bound to monomeric histone (Cantor & Schimmel, 1980). In contrast, Dieterich et al. (1979) found little change in rotational relaxation time before 0.35 M NaCl for the same fluor attached to Cys-110 of H3. Above this salt concentration, the rotational relaxation time decreased rapidly to 6 ns by 0.6 M NaCl. We suggest, therefore, that the H4 site remains associated with the octamer structure while the H3 site becomes unfolded and exposed to the aqueous environment at high salt concentrations. At 0.6 M NaCl, the histone octamer is bound to the nucleosomal DNA although there is a significant amount of reversible dissociation (Dieterich et al., 1979; Wilhelm & Wilhelm, 1980; Yager & van Holde, 1984; Ausio et al., 1984). At these relatively high ionic strengths, this dissociation behavior obviously complicates the interpretation of the fluorescence data as the properties of the various entities and their concentrations would need to be included in the analysis.

Binding of HMG 14/17 to Labeled Nucleosomes. The high mobility group (HMG) proteins 14/17 have received considerable attention because of their reported association with nucleosomes from transcriptionally competent chromatin (Weisbrod, 1982a,b). Mixing these proteins with core particles results in the formation of a complex containing two HMG 14 or 17 molecules per octamer (Albright et al., 1980; Sandeen et al., 1980). At low ionic strengths (10 mM Tris–borate and 0.25 mM EDTA, pH 8.4), two molecules of HMG 14 or 17 bind noncooperatively to a core particle while at 10–20 times higher ionic strengths there is a high degree of cooperativity (Albright et al., 1980; Sandeen et al., 1980; Schroter & Bode, 1982). Uberbacher et al. (1982) report that on the basis of neutron scattering studies the binding of these proteins causes subtle changes in the superhelical conformation of the DNA. Major rearrangements of the histone core were ruled out, but it was suggested that the basic N-terminal histone tails might move inward from their presumed contacts with the DNA and form a more compact protein core.

Both HMG 14 and HMG 17 proteins used were electrophoretically homogeneous. Five percent polyacrylamide particle gels established that binding to core particles had occurred. Competition experiments showed that there are no detectable differences in the binding of HMG 14/17 to native and labeled core particles (data not shown). The fluorescence emission intensities of 8-AEDANS Met H4-labeled core nucleosomes at two different ionic strengths with increasing equivalents of HMG 14/17 are shown in Figure 8a. During this titration experiment, aliquots were removed from the fluorescence cuvette to establish by electrophoresis that complexation with the labeled nucleosome had indeed occurred in the sample being examined. Binding studies were done with HMG 14, HMG 17, and also with an equimolar mixture of

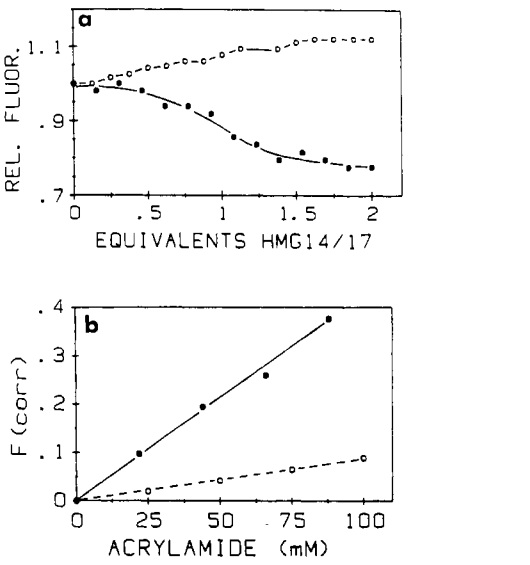

FIGURE 8: Effect of HMG 14/17 on the fluorescence emission of AEDANS-labeled nucleosomes. (a) Titration of AEDANS nucleosomes with HMG 14/17 in 10 mM Tris and 0.7 mM EDTA, pH 8 (○), and in 100 mM Tris–borate and 2.5 mM EDTA, pH 8 (●). (b) Stern–Volmer plots of labeled nucleosomes in 10 mM Tris and 0.7 mM EDTA, pH 8 (○), and in 100 mM Tris–borate and 2.5 mM EDTA, pH 8 (●), complexed with two molecules of HMG 14/17 per nucleosome. HMG 14, HMG 17, and equimolar mixtures of HMG 14/17 gave the same results. Excitation at 350 nm, emission at 480 nm.

HMG proteins 14 and 17. All gave the same results for the same amount of protein.

For the H4 Met-labeled nucleosome at low salt (10 mM Tris and 0.7 mM EDTA, pH 8), there was a monotonic increase in the fluorescence emission intensity until about 1.6 equiv of HMG 14/17 had been added. Beyond that point, the fluorescence emission remained constant, even when 3 equiv of HMG 14/17 per nucleosome was present. Particle gel analysis showed that while nonspecific binding of additional HMG molecules occurred, these extra ligands did not affect the fluorescence emission intensities. The binding of two HMG 17 molecules results in a decrease in the polarity around the label as reflected by an increase in emission intensity. Acrylamide quenching shown in Figure 8b for the 2 HMG 14/17–core nucleosome complex at this ionic strength reveals a 2–3-fold decrease in fluor accessibility from 10×10^{-7} to 4×10^{-7} mol^{-1} s^{-1}. Both of these results are consistent with a compaction of the protein environment around the fluor when two or more molecules of HMG 14/17 bind to the labeled nucleosome.

When the HMG 14/17 titration of labeled nucleosomes was performed at higher ionic strengths (100 mM Tris–borate and 2.5 mM EDTA, pH 8), very different results were obtained. The emission intensity decreases, and the transition is considerably more cooperative. Acrylamide quenching confirms that the label has become more accessible to solvent ($k_q = 19 \times 10^{-7}$ mol^{-1} s^{-1}), and thus at this higher ionic strength, the binding of HMG proteins appears to be characterized by expansion rather than compaction of the histone octamer. When 2 HMG 14/17–labeled core nucleosome complexes were titrated from low salt to high salt, the fluorescence emission did not decrease (data not shown). This result suggests that binding of the HMG proteins to a core nucleosome might induce conformations which are no longer sensitive to salt-induced structural changes. A similar property has been observed for histone H1 which is able to block the low-salt transition of nucleosomes (Burch & Martinson, 1980).

Further experiments are in progress to examine this phenomenon in more detail.

Discussion

We have reported the fluorescence emission characteristics of a covalently bound fluor at Met-84 on histone H4 which has been reconstituted into a core nucleosome. Similar studies have been made by others on Cys-modified H3 (Dieterich et al., 1977, 1979; Eshaghpour et al., 1980; Dieterich & Cantor, 1981; Daban & Cantor, 1982). One of the prerequisites for useful labeling is that the bound label not perturb the structure being studied. In the absence of a bioassay, we have applied most of the physical criteria which in other instances have allowed the detection of changes in the structure and stability of the nucleosome. The Met-labeled H4-containing nucleosome displays no detectable structural differences from a native nucleosome in contrast to the Cys H3-labeled particle. We do not mean to imply that the Cys H3-labeled nucleosome is structurally altered in its most stable state (i.e., 10 mM NaCl) but in other environments the structural destabilization resulting from the modification might possibly yield misleading results.

The environment-sensitive fluor AEDANS, located at a benign locus in the nucleosome, allows a detailed analysis of the conditions which in one way or another affect this site. Our measurements indicate that the polarity of the environment around Met-84 in H4, the accessibility to the quencher acrylamide, and the mobility of the fluor depend on the solution ionic strength as well as the binding of HMG 14/17. Thus, it is possible to delineate and examine the conditions which might allow changes in the conformation of the nucleosome. An understanding of these changes at the molecular level might be useful, for example, for understanding the structural basis of DNase I sensitivity of nucleosomes obtained from transcriptionally competent genes.

The behavior of the fluor at Met-84 on H4 in the nucleosome indicates that the salt-induced structural changes which occur do not result in the exposure of the fluor to the bulk solvent or unrestricted rotation. The emission properties change by less than 25% in the range 0.1–1000 mM NaCl and are characteristic of a fairly hydrophobic environment (Hudson & Weber, 1973). While the rotational mobility of AEDANS is essentially invariant for ionic strengths in the range 10–100 mM NaCl, the accessibility of the fluor to the quencher acrylamide changes considerably in the same range. Taken together, the emission and quenching results indicate the existence of five or six discrete states between 0.1 and 600 mM NaCl, all of which are probably folded, at least around Met-84 on H4. Interestingly, the binding of HMG 14/17 seems to be accompanied by both decreases and increases in accessibility depending on the initial ionic strength conditions. Since recent evidence has shown that HMG proteins 14/17 are unlikely to possess primary effector roles during transcription (Seale et al., 1983), we speculate that these proteins may be able to maintain and even regulate the formation of altered chromatin conformations by variable interactions with the nucleosome.

As mentioned earlier, there are conflicting reports in the literature regarding the unfolding of the protein core of nucleosomes at low and high ionic strengths. Evidence for histone core octamer unfolding derives from fluors bound to Cys H3 (Dieterich et al., 1977, 1979), from overall tyrosine emission and polarization (Libertini & Small, 1981), from chemical modification (Burch & Martinson, 1981), and from cross-linking (Burch & Martinson, 1980; Martinson et al., 1979) data. Contrary indications come from measurements of the nucleosome by sedimentation (Ausio et al., 1984) and neutron diffraction (Uberbacher et al., 1983). The changes observed, it has been argued by the latter researchers, involve mainly loosening or dissociation of the DNA ends from the histone octamer.

Our results from AEDANS-labeled H4 core nucleosomes are consistent with the idea that only comparatively small structural changes occur in the histone octamer over a very wide range of ionic strengths. We propose that the polarity, quenching, and mobility changes which occur with ionic strength and HMG binding of a fluor bound at Met-84 on H4 are the result of global alterations in the compactness of the histone octamer. The considerable amount of space not occupied by protein within the nucleosome (Klug et al., 1980; Richmond et al., 1984) could easily accommodate significant changes in the diameter and hence surface available for histone–DNA contacts. In view of the many DNA contacts with the globular portion of the histone octamer (Richmond et al., 1984), an internal structural change in the octamer could have a profound effect on the exterior of the histone octamer and its interaction with the supercoiled DNA. These changes in the core histone octamer might be induced or stabilized by non-histone protein binding, posttranslational modifications to the N-terminals of core histones, and covalent conjugation of ubiquitin to histones H2A and H2B, as well as DNA sequence dependent effects. Such a change in the nucleosome composition might, for instance, result in one state or other states shown in Figure 5 becoming the one of lowest free energy and thereby constitute a first step toward the attainment of transcriptional competence. Clearly, further studies are needed to establish whether, in fact, the histone octamer changes its volume and to assess what effect longer DNA or adjacent nucleosomes might have on these transitions. These questions form the basis of our ongoing studies.

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