Conformation of the HMG 14 nucleosome core complex from flow birefringence

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ABSTRACT

Flow birefringence and extinction angles have been measured for HMG 14 complexes with nucleosome core particles from chicken erythrocytes under cooperative "tight" binding conditions, and for the uncomplexed core particles used in the preparations. Results are interpreted using optical models for the observed DNA anisotropy, and are compared to recent small angle neutron scattering results. (19) The studies effectively rule out highly distorted DNA conformations and configurations in which DNA ends are unwound and extended. It is concluded that the most likely conformation of the complex is one in which the DNA superhelix is radially increased, either uniformly or bilaterally, with the DNA ends remaining tightly bound to the particle. This conformation does not require large changes in spatial relationships between the DNA ends compared to the uncomplexed core as would accompany, for example, significant unwinding of the ends. However, it may lead to more subtle but possibly highly significant differences in the angles at which the DNA exits the core particle.

INTRODUCTION

The 146 bp nucleosome core has been shown to be a constant and ubiquitous level of eukaryotic chromatin structure. (1,2) The general features of its structure are now known with considerable certainty through X-ray crystallographic studies (3,4) and image enhanced electron microscopy of core histone fibers. (5) Additional information upon its conformation and structure in solution has been obtained from neutron scattering experiments (6,7) and from electric (9,10) and flow (10-12) dichroism and birefringence. These and other physical properties have been recently reviewed. (2,13)

The high mobility group (HMG) nonhistone proteins bind to chromatin under physiological conditions and are widely distributed among eukaryotes. (14) Considerable presumptive evidence has accumulated that their presence is linked to transcriptional activity. (13,14) In particular, HMG 14/17 are thought to be structural proteins which may also play effector roles in active chromatin (13-16) since their presence is associated with enhanced DNase I sensitivity (15) and with transcribing gene sequences. (16,17)

HMG 14/17 bind reversibly to nucleosome core particles over a range of solvent ionic conditions. Strong, cooperative binding is observed at higher ionic strengths, (17,18) but the binding at low ionic strengths appears to be non-cooperative and thus perhaps may involve a different binding mechanism. A different mechanism also seems to apply for binding in excess of two proteins per nucleosome core. (18) Thus, the primary binding of HMG 14/17 seems to involve two equivalent binding sites (17,18) which may also be related by two-fold symmetry about the nucleosomal dyad axis. (18) No discernable differences between the binding of HMG 14 and HMG 17 have been reported. (18)

In a separate study from one of our laboratories, (19) small angle neutron scattering with H_2O/D_2O contrast matching has been used to study the complex formed under cooperative binding conditions between two HMG 14 molecules and chicken erythrocyte nucleosome cores. Using this technique, relatively accurate radii of gyration have been determined for both the DNA shell and the protein inner core of the complex, and these results have been interpreted in terms of several families of proposed structural variants. Radius of gyration results alone are insufficient to discriminate clearly among some of these variants, however, and significant functional differences may exist among several models having the same average radius of gyration. An independent and conformationally more sensitive way of distinguishing these would clearly be helpful.

In the present work, extinction angles and streaming birefringence data are reported for stoichiometric HMG 14 nucleosome core complexes under cooperative binding ionic strength conditions. Samples used are the same complex employed in the neutron scattering work. (19) Streaming birefringence provides conformational information on nucleosomes (10-12) which can be complementary to small angle neutron scattering and other methods that determine relatively accurately a single average molecular dimension such as a radius of gyration.

In these experiments, two types of structural information are obtained. Geometrical or shape anisotropy is obtained from extinction angles through the theory of rotational diffusion. A more important quantity, however, is the optical anisotropy from the birefringence since the birefringence can be determined experimentally at a somewhat higher level of accuracy. In favorable cases involving structures comprised of DNA, the anisotropy is extraordinarily sensitive to the specific DNA trajectory (10-12) and particularly to small changes in folding or unfolding. (11,12) Because of theoretical limitations in the interpretation of birefringence, the optical results by themselves do not constitute a satisfactory absolute structural method. (11) When combined with a relatively accurate radius of gyration from neutron scattering, however, consistencies

between the two methods assume importance since quite different physical principles are involved in the two measurements.

In this paper, we offer evidence based upon optical anisotropy and extent of rotational diffusion (i.e., orientation in a hydrodynamic velocity gradient) that the HMG 14 nucleosome core complex is in a highly compacted state similar to the uncomplexed core itself. The optical data are consistent only with configurations involving a small average increase in superhelical radius of the DNA supercoil and rule quite unambiguously against more extended models which involve even a limited disattachment or unwinding of the DNA ends.

METHODS

Mononucleosome cores from chicken erythrocytes and associated HMG 14 complexes were prepared as described elsewhere, (19) using established methods for both cores (12,20) and HMG 14. (21,22) Samples studied for this work were the same as used also for neutron scattering studies. (19)

All samples were unfrozen and were stored at 3°C prior to use. All studies used 0.3 x TBE buffer (0.0225 M tris, 0.6 mM EDTA and 0.0267 M borate, pH 8.3) prepared with glass distilled water. Both cores and complexes were run on particle and histone gels as described elsewhere. (12,19) Thermal denaturation profiles, sedimentation coefficients, optical absorption and circular dichroism spectra (the latter two from 220 to 300 nm) were all comparable to published results. (17) Small differences in circular dichroism spectra were observed between native cores and complexes.

The absolute viscosity of pure buffer was determined relative to water at 25°C in a long-capillary Ubbelohde viscometer: $\eta_0 = 0.892 \times 10^{-2}$ P. Solvent refractive index was measured in a Phoenix differential refractometer against standard KCl solutions as described earlier: (11) $n_0 = 1.3335$. Refractive index increments dn/dc were also determined by differential refractometry: (11) for the complex and core respectively, $dn/dc = 0.189 \pm 0.002$ and 0.184 ± 0.001 cm³/gm. The latter is in close agreement with a previous result from one of our laboratories for pure nucleosomes. (11)

The molecular weight of cores was taken as 2.11×10^5 daltons, (2) and that of the complex was calculated from published information (17) to be 2.31×10^5 for the 2-HMG 14 core complex. The partial specific volume of nucleosome cores has been given as $\bar{v} = 0.661 \pm 0.006$ cm³/gm at 10 mM KCl and 0.2 mM EDTA. (23) Uncertainty in this quantity is partly self-compensating in the present calculations, and in any event is relatively small compared to other sources of uncertainty. Moreover, it seems to be insensitive to ionic

strength at higher salt concentrations.(11,12) Hence, this value is used for pure cores in this work. Partial specific volume for the complex is obtained as follows. The results of the present study show that the overall conformational change in nucleosome cores as a result of binding 2 HMG 14 molecules is not large and that the particles can be represented as oblate disks or ellipsoids. The Scheraga-Mandelkern β -parameter is almost independent of axial ratio for oblate ellipsoids.(24) Using the experimental intrinsic viscosity data (Table I) and the sedimentation results of Sandeen et al. (17) in the Scheraga-Mandelkern theory, and assuming $\beta = 2.43 \times 10^6$ both for cores (calculated with $\bar{v} = 0.661$) and complex, we estimate $\bar{v} = 0.679 \text{ cm}^3/\text{gm}$ for the complex.

Intrinsic viscosity and flow birefringence data were obtained as in earlier studies.(10-12) The special photoelectric scanning flow birefringence instrument, its protocol of operation and the data reduction procedures have also been described.(10) Principal differences between this study and prior ones from this laboratory are as follows. The averaging of the photomultiplier signal was done digitally by computer using its internal time standard, and measurements were made at such values of the instrument angular variables as to provide both A and B signals (ref. 10) of maximum possible signal-to-noise. These changes resulted in improvements in precision, particularly in the measurement of extinction angles very near 45° , and were especially important here because sample concentrations were lower than in previous work due to limited sample availability. All curve fitting procedures employed a computer algorithm for a least-squares polynomial of up to sixth order. The number of terms actually used was determined by the quantity of data points available and by standard analysis of variance methods. All standard errors quoted in this work derive from such analyses unless otherwise noted.

A new, more adequately thermostatted concentric cylinder flow birefringence cell fabricated of stainless steel was used in the present work. This cell had an optical path length of 10 cm and an annular gap of 0.25 mm. All data reported were obtained well within the effective nonturbulent range of this cell. Short run times made possible by improved time averaging combined with long thermal re-equilibration times between shear point determinations ensured that negligible sample heating occurred in all cases. Hydrodynamic property and spectroscopic characterization of representative samples following flow birefringence runs established that the methods used in this work were entirely nondestructive to cores and complexes alike.

RESULTS AND DISCUSSION

Experimental extinction angle data extrapolated to zero concentration are shown in Fig. 1 for both cores and HMG 14 complexes. Results for the two are indistinguishable within experimental scatter. Concentration dependence of the extinction angle for both samples (data not shown) is small over the sample concentration ranges used here.

These results are not sufficiently precise to determine reliable diffusion coefficients, but they agree with earlier determinations on nucleosomes (11,12) within experimental error. This is illustrated by comparing extinction angle data with the theoretical curves assuming a rotational diffusion coefficient $D_r = 4.2 \times 10^5 \text{ sec}^{-1}$ (11) and prolate or oblate symmetry with axial ratios $p = 2$ (dashed line) or $p^{-1} = 2$ (solid line) respectively. From this analysis, it is evident that the axial ratios and rotational diffusion coefficients for cores and complex are the same within the necessarily rather large experimental error, and are approximately given by the above values.

Flow birefringence curves as functions of shear rate G are shown in Fig. 2. The reduced birefringence or Maxwell constants, $[n] = (\Delta n/cG)_{c,G \rightarrow 0}$, shown in Table I are obtained from these data by determining limiting slopes (linear term coefficients in a least squares polynomial) of Δn vs G and $(\Delta n/G)_{G \rightarrow 0}$.

Figure 1. Experimental extinction angles in degrees extrapolated to zero concentration (χ) vs velocity gradient (G) for chicken erythrocyte core particles and HMG 14 core particle complexes. Solid circles ●: HMG 14 core particle complex. Open circles ○: pure core particles. Theoretical curves for $D_r \approx 4.2 \times 10^5 \text{ sec}^{-1}$ (see ref. 11). Solid line assuming oblate particles with axial ratio $p^{-1} = 2$. Dashed line assuming prolate particles with $p = 2$.

Figure 2. Experimental flow birefringence data (Δn) as a function of velocity gradient (G) for chicken erythrocyte core particles and HMG 14 core particle complexes. Solid circles $\bullet$: HMG 14 core particle complex. Open circles $\circ$: pure core particles. Concentrations (from top down). Complex: 111 (5 runs), 88 (1 run), 68 (3 runs), 47 (3 runs) and 38 (3 runs) $\mu\text{gm/ml}$. Cores: 178 (3 runs), 117 (1 run), 105 (3 runs) and 71 (3 runs) $\mu\text{gm/ml}$.

vs concentration c (shown in Fig. 3) or of intercepts of $\Delta n/G$ vs G and $(\Delta n/cG)_{G \rightarrow 0}$ vs c plots; since these plots are essentially linear, both representations are nearly equivalent, and the results reported in Table I are calculated using the first representation because it gave lower statistical errors. However, this comparison shows that extrapolation errors are negligible in the present work. Statistical errors quoted for $[\eta]/[\eta]$ in Table I are due primarily to estimated uncertainty in the measurement of intrinsic viscosity. The latter in turn derives mainly from errors in concentration measurement. If this is systematic, such errors should largely cancel in the ratio $[\eta]/[\eta]$ and the quoted uncertainties are upper limit values.

In earlier studies, (11,12) it has been shown that the optical anisotropy ($g_a - g_b$) (eq. 7, ref 11 or eq. 1, ref. 12), or equivalently, the apparent intrinsic birefringence $\Delta n_{\text{int}} = n_a - n_b$ obtained using the theory of macroscopic continuum dielectrics as described (11) is an extremely sensitive function of DNA trajectory in a DNA-containing ultrastructure such as a nucleosome. This is because of the high optical anisotropy of bihelical DNA itself; the DNA contribution to the overall anisotropy is large in comparison to that from proteins or other relatively more isotropic components. (11) The

TABLE I

	Core Particles	HMG 14 Complexes
dn/dc	$0.184 \text{ cm}^3/\text{gm}$	$0.189 \text{ cm}^3/\text{gm}$
$\bar{v}$	$0.661 \text{ cm}^3/\text{gm}$	$(0.679)^a \text{ cm}^3/\text{gm}$
n_o^b	1.3335	1.3335
η_o^b	$0.890 \times 10^{-2} \text{ P}$	$0.890 \times 10^{-2} \text{ P}$
M^c	$2.11 \times 10^5 \text{ daltons}$	$2.31 \times 10^5 \text{ daltons}$
$[\eta]$	$12.0 \pm 1.2 \text{ cm}^3/\text{gm}$	$13.1 \pm 1.3 \text{ cm}^3/\text{gm}$
$S_{20,w}^c$	11.1 svedbergs	10.8 svedbergs
$[n]^d$ (cgs units)	$-1.139 \pm 0.001 \times 10^{-7}$	$-1.036 \pm 0.001 \times 10^{-7}$
$\frac{[n]}{[\eta]}$ (cgs units)	$-9.495 \pm 0.01 \times 10^{-9}$	$-7.912 \pm 0.01 \times 10^{-9}$

Experimental physical quantities determined from the present work.

a Calculated (24) from $[\eta]$ and $S_{20,w}$ assuming $\beta = 2.43 \times 10^{-6}$ (see text).

b $0.3 \times$ TBE buffer.

c Reference 17 and information therein.

d Error limits from concentration dependence data only (see text).

e Error limits assumed determined by intrinsic viscosity (see text).

Figure 3. Concentration dependence of $(\Delta n/G)$ at $G \rightarrow 0$ (in cgs units) for $\bullet$ HMG 14 core particle complexes and $\circ$ for pure core particles.

measured optical anisotropy can therefore be interpreted in terms of "optical" models of various DNA conformations. It is usually not possible to make absolute or a priori optical calculations of this type because of the widely noted inadequacies and imperfections in the classical theory of birefringence. (11,25-28) However, this is not a serious limitation in the present context because the experimental optical anisotropy of linear, bihelical DNA is known with some confidence, (26) and the anisotropies of model structures can be determined in relation to this. Such analyses seem to be considerably more sensitive and less ambiguous than the interpretation of hydrodynamic properties alone, and additional constraints are imposed on the optical models by the requirement that they must be consistent geometrically with rotational diffusion coefficients estimated from the extinction angle data. In this work, further constraints are imposed by the accurate radius of gyration of the DNA component available from small angle contrast matching neutron scattering studies. (19)

Using the methods developed previously (eqs. 7-10 of reference 11), the experimental intrinsic birefringence is obtained from (dn/dc) and $[n]/[\eta]$ values of Table I. Because of the anisometry dependence of certain quantities in the theory, the experimental intrinsic birefringence can only be obtained as a function of axial ratio. Hence, all comparisons are made on this basis. For comparison with experimental results, three general classes of optical models are considered (Fig. 4), and the representative optical properties calculated for these are also shown in Figs. 5. The principal assumptions are that classical macroscopic continuum dielectric theory applies in the same relative way to particle-bound and free DNA, and that the particles have, or time average to under the dynamics of motion in a velocity gradient, ellipsoidal (or approximately cylindrical) symmetry.

The first of these assumptions is largely obviated in comparing experimental results with optical models based on DNA trajectories since the same experimental and theoretical principles used to estimate the intrinsic birefringence of DNA (11,26) are also used in the model calculations. The second assumption is based upon rigid particle hydrodynamic theory (29) and empirical observations of macroscopic systems. (30) The unfolded core particle forms (Models X and C of Fig. 4) have relatively low symmetry, and it is not in general clear how these would actually orient in a shear field. Specifications of "oblate" or "prolate" particle symmetry are therefore based upon assumed effective orientation, i.e., upon choice of major axis with the spin component about this axis (29,30) leading to quasi-cylindrical or ellipsoidal symmetry and the minor axis determined as the average of the two remaining particle dimensions. We do not propose that such

Figure 4. Schematic representation of DNA conformations used in optical model analyses with figures indicating the (approximate) axis of the DNA double helix. Model B represents a uniform DNA superhelix of oblate overall symmetry. Models X and C represent partially unwound states of the basic nucleosome core. Model X has 10 bp DNA unwound at either end, assumed straight; the orientation of these unbound tails is determined by the dihedral angle ϕ and lateral extension in the direction of the superhelical axis by angle θ . Model C has 32 bp DNA unbound and assumed straight and retains one full superhelical turn about the octameric histone core; orientation of the tails in this case is determined by dihedral angle ϕ and core twist angle θ (see also ref. 12).

specifications necessarily describe the true shape and symmetry of the particle; only the effective hydrodynamic shape. As in a previous study, (12) we have examined the various prolate forms of the models shown and have found no correspondence with experiment. Hence, only the oblate forms are shown and discussed here.

Model B is a uniform DNA superhelix around the core histone octamer. It has been described and its properties discussed elsewhere. (11,12) For simplicity, we assume that the total volume is conserved in all cases throughout the range of axial ratios investigated at the same value as the 55×110 Å oblate disk model of Finch et al. (3) Although the effective volume of the HMG 14 complex from neutron scattering is increased about 10% over that of the core, (19) and our own calculations imply an increase in partial specific volume of about 3% (Table I), the constant volume approximation does not significantly affect the conclusions to be drawn here.

Figure 5. Intrinsic birefringence vs axial ratio for oblate particles in the shear field. (a) Experimental curves, least square polynomial fits to data points (not shown): upper curve, core particles; lower curve, HMG 14 core particle complex. (+) Model X calculation for $\phi, \theta = 0^\circ, 10^\circ, 30^\circ, 60^\circ$ and 90° . (O) Model C calculation for $\phi, \theta = 0^\circ, 20^\circ, 40^\circ, 60^\circ$ and 80° . Heavy solid curve: constant volume uniform superhelix model B (see text). (b) (●) Experimental data points for HMG 14 core particle complexes. (O) Experimental data points for pure core particles. Curves through data points are least square polynomial fits. Solid curve: constant volume uniform superhelix Model B (see text).

Within the concept of the uniform DNA superhelix, two subclasses can be distinguished as a consequence of the constraint of fixed DNA contour length upon changes of overall axial ratio: (1) a model in which superhelical pitch

is held constant and the number of superhelical turns varies with axial ratio; and (2) a model in which the number of turns is held constant at the core particle value of 1.75 and helical pitch varies with axial ratio. These correspond respectively to Models I and II of a previous report;(11) hence, Δn_{int} can be obtained for either one as a function of axial ratio using eqs. 12,16 and 17 of ref. 11 as described, and the experimental (flow birefringence) value for pure DNA $\Delta n_{\text{int}} = -0.1268$.(26) The first case involves a simple winding or unwinding of the DNA as the axial ratio of its supercoil is changed. Such a model would seem reasonable in the present context since the DNA "pathway" around the core histone octamer would remain invariant. The second model more closely preserves the DNA core histone binding sites. The latter do not seem to be particularly specific at the core particle level, however, and the ability of cores to slide or translate on DNA is well known.(1,2) Hence, the Model B curves shown in Figs. 5 are for the variable turns assumption with the effective superhelical radius of the DNA reduced 10 Å over the radius of the overall equivalent cylindrical volume. We note that over a small range of oblate axial ratios around $p^{-1} \approx 2$ (the range of interest here), the two are optically almost equivalent. However, if DNA conformation changes by the first mechanism, both the relative positions and exit angles of the DNA ends in the core particle will depend upon axial ratio.

Models X and C allow for various degrees of DNA unwinding from the ends. Other studies have shown that HMG 14/17 binding to nucleosome cores involves the first 10-20 bp from the DNA ends.(17,18) In model X, 10 bp from each DNA end is unwound and assumed straight.(12) The orientations of these ends are at an angle ϕ with respect to the tangent to the coil at the point of DNA attachment, and θ with respect to the superhelical axis; for a given θ , the ends are assumed to point in opposite directions. This model contains 1.5 superhelical turns of DNA. Model C (see also ref. 12) is a more unwound variant containing 1 superhelical turn of DNA and straight, unbound ends ~ 30 bp in either direction. Orientation of the ends with respect to the superhelical coil is determined by the dihedral angle ϕ and core twist angle θ as shown in Fig. 4.

The question of modeling unbound DNA as straight segments has been considered earlier.(12) We assume that unbound nucleosomal DNA is conformationally similar to free DNA in solution. The stiffness of the DNA helix in solution is characterized by the persistence length, which at ordinary temperatures is around 50 nm.(28) In free solution, therefore, DNA bends (by thermal forces) through a mean radius of curvature of ~ 42 nm, or about 8-fold greater than in a nucleosome core. This degree of curvature leads only

to minor effects upon the optical properties of models C and X, and we therefore feel that it can be neglected in relation to other uncertainties inherent in the optical descriptions of these models. Moreover, such bending effects will be largely averaged out over a population to the degree that they are thermal in origin. Finally, since the experimental intrinsic birefringence of DNA was obtained (by flow birefringence) upon approximately 1 persistence length DNA, (26), the thermal bending effect upon the optical anisotropy of free DNA is already formally included in that quantity.

Calculated intrinsic birefringence values for Models X and C are shown in Fig. 5a, corresponding to various pairs of the angular variables. Calculations shown are for all combinations of the angular variables $\phi, \theta = 0^\circ, 10^\circ, 30^\circ, 60^\circ$ and 90° for Model X and $0^\circ, 20^\circ, 40^\circ, 60^\circ$ and 80° for Model C. Details of these calculations have been discussed previously. (12) Axial ratios are based upon the overall dimensions of the resultant conformations assuming effective oblate symmetry as discussed above. It can be seen that all Model X variants lie well off the experimental curve at all axial ratios. A few Model C conformations are commensurate with the experimental lines, but these are all variants of $\phi < \sim 20^\circ$ with $\theta > \sim 70^\circ$. These have axial ratios $p^{-1} \approx 2$ because of the large twist angle θ , and hence are not ruled out by the extinction angle data; such extended conformations would lead to radii of gyration much larger than observed. (19) On the other hand, the oblate superhelix Model B crosses the experimental curves at axial ratios $p^{-1} \approx 2$ (see also Fig. 5b), which is consistent with the extinction angle data of Fig. 1.

Neutron scattering results (19) show increases in radius of gyration of 2.7 Å for DNA and 0.9 Å for protein in HMG 14 binding to nucleosome cores. Families of models most consistent with these results include uniform radial expansion of the DNA superhelix and elliptical or bilateral expansion of the DNA in the direction of the assumed protein binding sites. Several other types of models can be extended to fit the data but were thought unlikely on other grounds. These were inconsistent with DNase I digestion results and sedimentation data in the context of the measured protein radius of gyration. They include expansion modes involving either DNA hinging at the center or unwinding and reorientation of the DNA ends. The latter are clearly ruled out by the present analyses of Models X and C. The optical properties to be expected from a lateral unfolding at a central point in the DNA, i.e., an "opening clam-shell" type of model, should lead to a numerical increase in $-[n]/[\eta]$, whereas a decrease is actually observed in going from core to complex (Table I). Similarly, an axially expanded uniform superhelix consistent with the observed radius of

gyration would have a DNA end-to-end distance of about 87 Å. (11) If particle volume is conserved to at least 10%, this model approaches an axial ratio $p^{-1} \approx 1$ and should exhibit far less orientation under the shear conditions of this study than implied by the observed extinction angles (Fig. 1). The present results are therefore most consistent with Sandeen et al.'s observations (17) that the premelting transition normally observed in the nucleosome core is lost with stoichiometric HMG 14/17 binding. This premelting transition has been associated with loosening of the DNA ends. (2,13,31)

Although the optical modeling seems clearly to rule against more extended complex conformational possibilities, it is difficult to discriminate between uniform and non-uniform or bilateral radial expansion in the DNA from the present results. In Fig. 5b, the experimental Δn_{int} vs p^{-1} curves for cores and complex are distinct within the range of experimental uncertainty given by the standard errors in Table I (standard error in Δn_{int} is about $\pm 3 \times 10^{-5}$). This, of course, neglects the uncertainty in $\bar{v}$, particularly for the complex. However, if it is assumed that radial expansion is uniform and if these curves are taken at face value, they reflect an increase in p^{-1} from about 2 to 2.2 or roughly a 10% increase in effective particle radius with complex formation. This is slightly larger than the 6% increase obtained from neutron scattering, (19) but within limitations of the present method, the agreement is entirely satisfactory.

Bilateral radial expansion of the DNA supercoil might occur either through a sideways or lateral translation of one (1.75/2) turn of the supercoil with respect to the other, or through an elongation in the direction of protein binding since strong presumptive evidence exists that the binding sites are symmetrically arranged with respect to the nucleosomal dyad. (17,18) To be consistent with the observed radius of gyration, the first of these requires a displacement of ~ 18 Å and the second a radial elongation of 6 to 8 Å, depending upon assumptions about the DNA ends. (19) The expected change in Δn_{int} for the 18 Å lateral supercoil translation case can be estimated using eq. 12 and the methods described in ref. 12, and is found to increase in a positive sense for an oblate model if major axis and supercoil pitch are kept approximately the same. This is a change in the opposite direction to that observed experimentally at all axial ratios (Fig. 5b). Furthermore, it is difficult to rationalize this model with conservation of histone core radius since it would evidently require some disruption of the normal octameric structure. We have not investigated the optical properties of an elliptical superhelix, but a simple extension of the Model X calculation to include the contribution of an 8 Å region of linear DNA in the direction of bilateral expansion (a torus or "racetrack" configuration) leads to the same result.

These conformational changes are subtle but they may be quite important if they are related to structural differences between active and bulk chromatin. In this connection, it seems significant that the unbound end models are of relatively low probability, and that particle expansion with HMG 14/17 binding evidently occurs radially. Furthermore, the evidence presented here, although not definitive, is nevertheless highly suggestive that this radial expansion is largely uniform.

The question of specific model assumes considerable importance in terms of HMG 14/17 binding effects upon higher order structure since the different models may lead to different exit directions of the DNA ends. The neutron scattering results (19) show only a small increase in protein radius which may be accounted for by the additional HMG 14 molecules themselves. However, the DNA expansion consistent with both the neutron work and the present results, if assumed uniform, leads to a reduction in superhelical turns with HMG 14/17 binding from 1.75 to ~ 1.65 , corresponding to a change in relative DNA exit angle from 90° to $\sim 55^\circ$. Qualitatively similar results obtain for the bilateral radial expansion models, but in this case, the range of possible exit angles is larger and depends upon the assumed spatial relation between the HMG 14/17 binding sites and the DNA ends.

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