An Approach to the Structure of Native Nucleohistone†

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ABSTRACT: We have studied the circular dichroism (CD) spectra of nucleohistone in a series of solvent systems which are known to change the hydrodynamic properties of nucleohistone solutions. The hydrodynamic changes are characterized by large increases in viscosity. The CD spectral shifts in these solvent systems invariably display an increase in ellipticity in the region 260–300 mμ (though of variable extent), so that the CD spectra in the region more closely resembles that of free DNA. We have interpreted the changes in hydrodynamic properties and CD spectra as reflecting a change in the compact, supercoiled nucleohistone structure toward a more extended form. Since the solvent systems change the nucleohistone structure in different ways (urea affects protein secondary structure, low ionic strengths increase the effectiveness of the negative charge on the nucleohistone backbone and intermediate ionic strengths should drastically weaken histone–DNA electrostatic bonds) we have had the opportunity to analyze the different forces that compel nucleohistone to adopt a compact conformation. A hypothetical model for nucleohistone structure based on these studies is presented.

A number of hydrodynamic studies have indicated that nucleohistone molecules in solution exist in a more compact conformation than free DNA (Simpson, 1972; Bayley *et al.*, 1962; Chalkley and Jensen, 1968; Ohba, 1966). This concept has been extended with the technique of X-ray diffraction to show that molecules in bovine thymus nucleohistone fibers are in the form of regular supercoils with a pitch of 120 Å (Pardon *et al.*, 1967).

Little is known concerning the nature of the forces which are involved in maintaining the supercoiled conformation. We have approached this problem by looking at systems which grossly change hydrodynamic properties of solutions of nucleohistone. The systems studied have been (1) elevated urea concentrations, (2) very low ionic strengths (<0.001), and (3) intermediate ionic strength (0.6 M NaCl). In all cases exposure of nucleohistone to these environments gives rise to a large increase in viscosity without dissociation of histone, except for 0.6 M NaCl which removes the F_1 histone fraction. We have correlated changes in hydrodynamic properties with circular dichroism (CD) spectra in an attempt to extend our understanding of the forces involved in the maintenance of the compact, supercoiled conformation of nucleohistone. In these studies we have been aided by earlier work which has described the CD spectrum of chromatin (Shih and Fasman, 1970; Simpson and Sober, 1970; Henson and Walker, 1970) and related the decreased elliplicity of DNA in nucleohistone in the region 260–290 nm to the supercoiled conformation which it has adopted within the nucleoprotein (Shih and Fasman, 1970, 1971; Olins and Olins, 1971; Wagner and Vandegrift, 1972).

We have also studied the circular dichroism of nucleohistone from which specific histones have been removed. Several other investigators have looked at the optical properties of partially dissociated nucleohistone and although they are in agreement that histones associated with DNA in the nucleohistone complex most likely induce a base tilting upon formation of a supercoiled structure of the DNA (which is indicated by a change in the circular dichroism), they disagree as to which histone fractions are making the greatest contribution to maintaining the supercoiled structure of nucleohistone. Henson and Walker (1970) suggest that histone fractions F_3, F_{2b}, and F_{2a2} maintain the supercoiled structure of nucleohistone, Simpson and Sober (1970) favor fractions F_{2b} and F_{2a2}, Wagner and Spelsberg (1971) prefer histone F_{2a1}, and Tuan and Bonner (1969) have more cautiously suggested it is not histone F_1 or the non-histone proteins.

These investigators were not aware of or have ignored the problems which accompany the dissociation of histones from nucleohistone with sodium chloride (Bartley and Chalkley, 1972). The residual nucleohistone may be contaminated with dissociated histones (through the aggregation and subsequent sedimentation of many of the dissociated histones). Furthermore extensive proteolysis of the histones of the residual nucleohistone could influence the hydrodynamic and optical properties of such material. For these reasons we have reinvestigated the circular dichroism of partially dissociated nucleohistone which was prepared in conditions where aggregation of dissociated histones and proteolysis do not occur.

Materials and Methods

Preparation of Nucleohistone and DNA. Nucleohistone from bovine thymus, bovine lung, or chicken erythrocyte was prepared and analyzed for DNA content as described previously (Panyim *et al.*, 1971). In order to obtain a soluble preparation of nucleohistone the chromatin was sheared in a Virtis homogenizer (30 V for 1.5 min) and the nucleohistone was clarified by centrifugation (12,000*g* for 20 min) and stored at 2°.

DNA of the same size distribution as that in nucleohistone was obtained by treating nucleohistone with sodium dodecyl sulfate to a final concentration of 1% (0.01 M Tris-HCl, pH 8.0) and sedimenting (100,000*g* for 12 hr) at 14° (sodium dodecyl sulfate precipitates at lower temperatures) into an underlayer of 20% sucrose, 1% sodium dodecyl sulfate, and 0.01 M Tris-HCl (pH 8.0) (2 ml). The pellet was rinsed twice and suspended in 1% sodium dodecyl sulfate (0.01 M Tris-HCl, pH 8.0) and dialyzed against 0.01 M Tris-HCl (pH 8.0).

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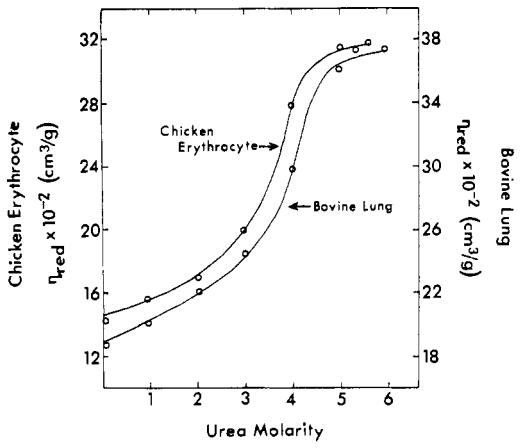

FIGURE 1: The dependence of the reduced viscosity of bovine lung nucleohistone and of chicken erythrocyte nucleohistone upon urea concentration. The urea solutions were buffered with 0.01 M Tris-HCl (pH 8.0). The concentration of bovine lung nucleohistone or chicken erythrocyte nucleohistone in terms of DNA content is 105 and 117 μg per ml, respectively.

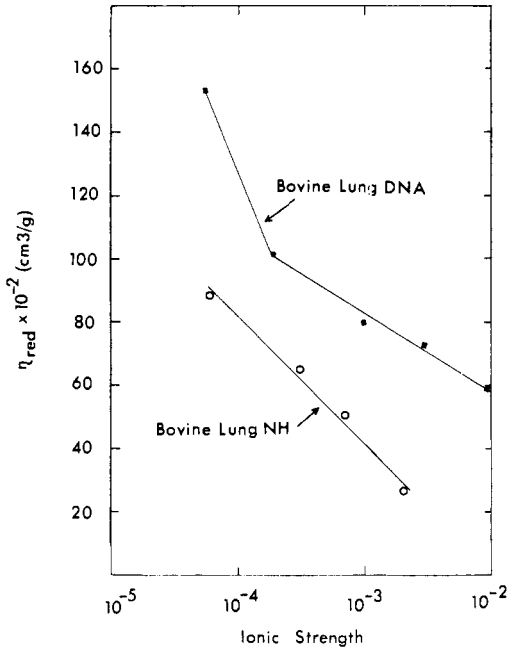

FIGURE 2: The reduced viscosity of bovine lung nucleohistone or bovine lung DNA as a function of decreasing ionic strength. The measurements were obtained at 2° in the appropriate Tris-HCl buffers (pH 8.0). The concentration of the nucleohistone (in terms of DNA content) was 132.0 μg/ml and of the DNA was 86.8 μg/ml.

Such preparations contain less than 7% protein, which can be reduced to less than 2% during a second purification cycle.

Preparation of Partially Dissociated Nucleohistone. Partially dissociated bovine thymus nucleohistones were prepared as described previously (Bartley and Chalkley, 1972).

Viscosity Measurements. Viscosity data were obtained at 5° using an Ostwald viscometer modified for low sheer forces. The temperature was regulated to ±0.01°. The solutions were filtered through sintered glass prior to use. All determinations, including those in urea solutions, were conducted at constant ionic strength using 0.01 M Tris-HCl (pH 8.0), unless otherwise noted. The solutions were prepared by diluting a stock

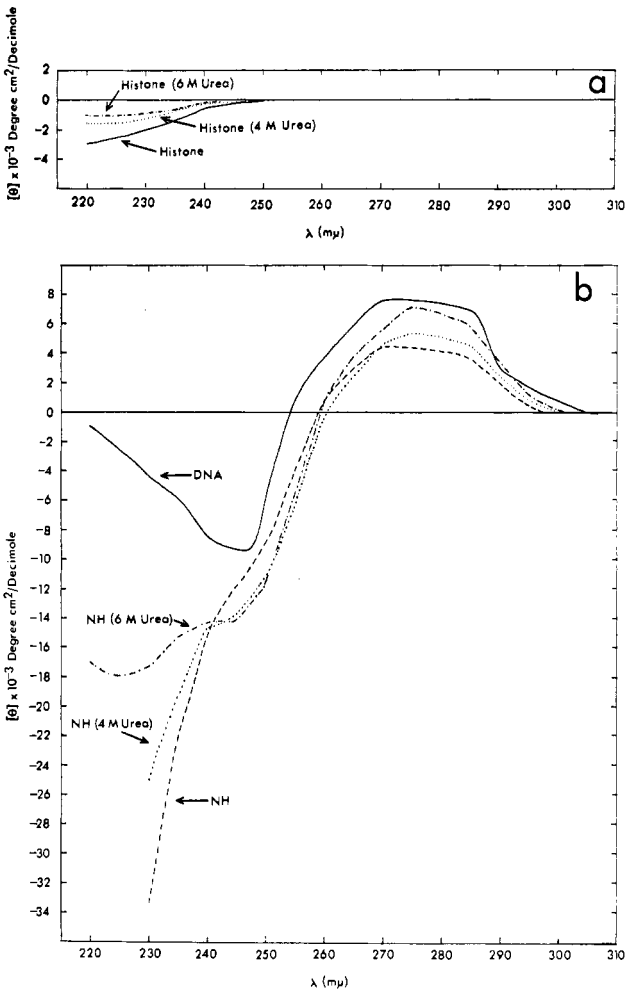

FIGURE 3: The effect of urea (4.0 and 6.0 M) upon the circular dichroism of (a) bovine thymus histones and (b) bovine thymus nucleohistone (NH). Bovine thymus DNA is shown in part b. All solutions were buffered with 0.01 M Tris-HCl (pH 8.0).

solution of nucleohistone just prior to determining its viscosity. The DNA content of the nucleohistone was calculated from the optical density at 260 mμ (Chalkley and Jensen, 1968). Urea solutions were freshly prepared immediately before use.

Circular dichroism was measured using a Cary Model 60 recording spectropolarimeter with a Model 6002 CD attachment. The solutions were prepared and analyzed as for the viscosity measurements. All experiments were carried out at 27 ± 0.5° in 1-cm or 2-mm cells using an $A_{260\ m\mu}$ of less than 1.0 for DNA and $A_{230\ m\mu}$ of less than 0.5 for histones. The mean residue ellipticity, $[\theta]$, is expressed in (deg cm²)/dmol of nucleotide residues or histone amino acid residues assuming the mean molecular weight of a nucleotide as 323 and of a histone amino acid as 100. In histone–DNA complexes, *e.g.*, nucleohistone and partially dissociated nucleohistone the mean residue ellipticity, $[\theta]$, is expressed per decimole of nucleotides. On occasion, where specified, the ellipticity is also expressed as (deg cm²)/dg of DNA, histone, or nucleohistone.

Results

Three Systems for Perturbing the Hydrodynamic Behavior of Nucleohistone. UREA SOLUTIONS. The effect of increasing concentrations of urea upon the reduced viscosity of calf lung and

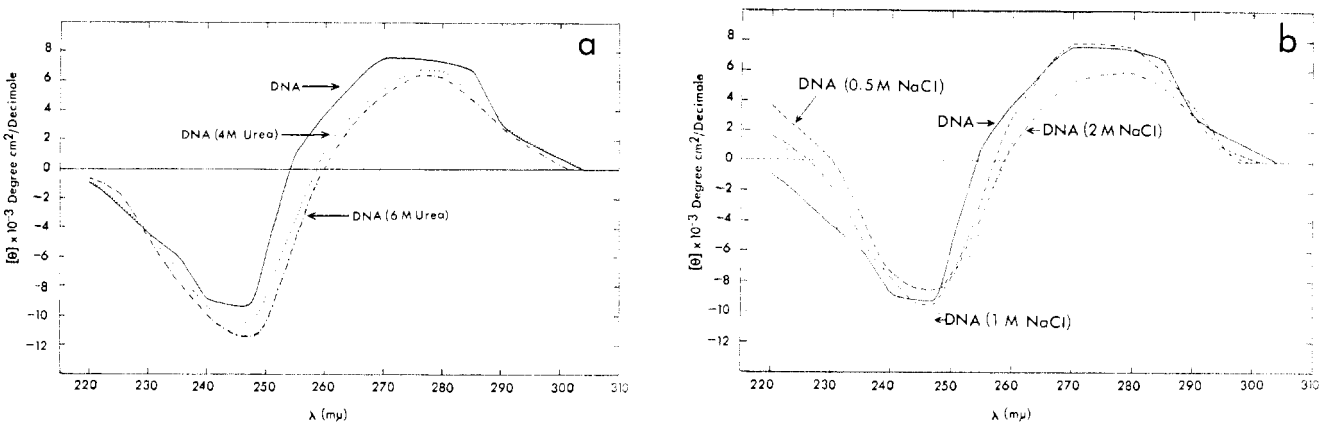

FIGURE 4: The effect of urea or sodium chloride upon the circular dichroism of bovine thymus DNA. The concentrations of (a) urea and (b) sodium chloride are indicated on the figure. All solutions were 0.01 M Tris-HCl (pH 7.0).

chicken erythrocyte nucleohistones is shown in Figure 1. In agreement with previous reports for calf thymus nucleohistone (Bartley and Chalkley, 1968) there is a rapid two- to threefold increase in viscosity as urea concentration increases. Evidently the ability to generate an increased viscosity in urea solutions is a general property of nucleohistone molecules. The viscosity increase is not due to removal of histones nor is it thought to be due to excessive levels of hydration by urea or water and we have interpreted the viscosity increase in terms of an extension of the nucleohistone molecule to a less compact form (Bartley and Chalkley, 1968).

VERY LOW IONIC STRENGTHS. It has been known for many years that viscous chromatin gels can be prepared by lowering the ionic strength evironment. The more simple nucleohistone structure responds in a similar fashion to ionic strength as is shown in Figure 2.[1] The viscosity behavior of DNA under the same conditions is also shown. The viscosity of DNA increases some 2.5-fold as the ionic strength is decreased from 10^{-2} to 10^{-5}. This is presumably due to the electroviscous effect and to changes in the extent of hydration of the molecule. Nucleohistone shows a ninefold increase in viscosity over the same range of ionic strengths. It is unlikely that this represents a greater electroviscous or hydration effect as the nucleohistone molecule has only approximately one-fourth of the negative charge density residing on the DNA molecule and it seems likely that the nucleohistone molecules are becoming more extended at the lower ionic strengths presumably due to an increased electrostatic repulsion along the nucleohistone backbone as the positive ion atmosphere is removed.

INTERMEDIATE IONIC STRENGTH (0.6 M NaCl). Extraction of nucleohistone in sodium chloride (0.6 M) removes the lysine-rich histone (F_1); the residual nucleohistone at an ionic strength of 0.01 has a viscosity behavior characteristic of native nucleohistone (Henson and Walker, 1970). However if the residual nucleohistone is analyzed by sedimentation velocity in 0.6 M NaCl (Chalkley and Jensen, 1968) then it behaves as though it is in the extended form showing a sedimentation constant similar to that observed in high concentrations of urea. Since 0.6 M sodium chloride should effectively abolish electrostatic interactions between DNA and histones, it seems likely that intact DNA–histone electrostatic bonds are an integral and necessary requirement for the compact conformation of native nucleohistone.

Circular Dichroism of Nucleohistone in Urea. The effect of urea upon the circular dichroism of bovine thymus nucleohistone is seen in Figure 3b. Urea causes the circular dichroism of nucleohistone to become more like that of DNA. Below 240 mμ the effect of urea upon the CD spectrum is most dramatic and the band becomes much less negative with increasing urea concentration. The band between 260 and 300 mμ becomes more positive (~80%) as the urea concentration increases.

The CD of bovine thymus histones is seen in Figure 3a. Histones do not contribute to the spectrum above 250 mμ which agrees with the observations of Fasman *et al.* (1970). Between 220 and 250 mμ the CD of histones becomes less negative as the urea concentration increases, though the extent of this decrease is much smaller than that observed for nucleohistone.

In contrast (Figure 4a) the CD spectrum of free DNA in urea solution shows a decrease in ellipticity in the region 260–300 mμ together with a small red shift. This spectral behavior is reminiscent of the behavior of DNA in solutions of increasing concentrations of NaCl (Figure 4b) and may have its origin in similar dehydration phenomena as previously suggested by other workers (Shih and Fasman, 1970). No matter what the origin of the urea-induced spectral shift for DNA it is in the opposite direction to that observed for the effect of urea on the nucleohistone CD spectrum.

Circular Dichroism of Nucleohistone at Low Ionic Strengths. The CD spectra of nucleohistone in very low ionic strengths and in the standard ionic strength (0.01, in which nucleohistone does not begin to precipitate) are compared in Figure 5. The effect of lowering the ionic strength is to increase the positive ellipticity in the region 260–300 mμ by approximately 20%, thus imitating the urea effect, though the magnitude of the change is appreciably less. There is relatively little change in negative ellipticity at lower wavelengths in the lower ionic strength environment.

Circular Dichroism of Nucleohistone Lacking F_1 at Various Ionic Strengths. Nucleohistone lacking F_1 is easily prepared by extraction in 0.6 M NaCl. Subsequently the CD behavior of such nucleohistone was compared in 0.6 M NaCl where electrostatic interactions should be minimal and in 0.01 M Tris-HCl at the same pH. As shown in Figure 6 the effect of 0.6 M NaCl is to increase the positive ellipticity by 60% so that it

[1] Nucleohistone is prepared by vigorously, physically disrupting the chromatin and is thus operationally defined. It does not form a solid gel in low ionic strengths, but nonetheless demonstrates an increase in viscosity.

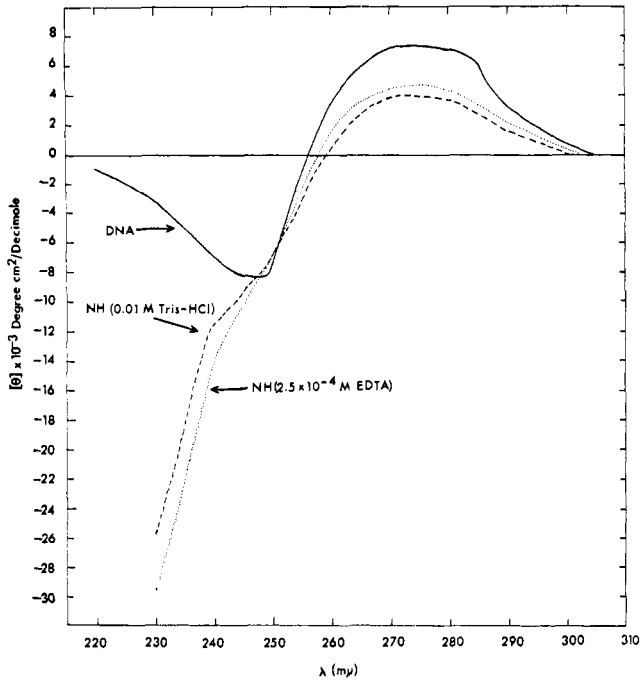

FIGURE 5: The circular dichroism of nucleohistone in different ionic strengths. (a) The CD of nucleohistone in 0.01 M Tris-HCl (pH 7.0) is compared to that of nucleohistone in low ionic strength (2.5×10^{-4} M EDTA, pH 7.0).

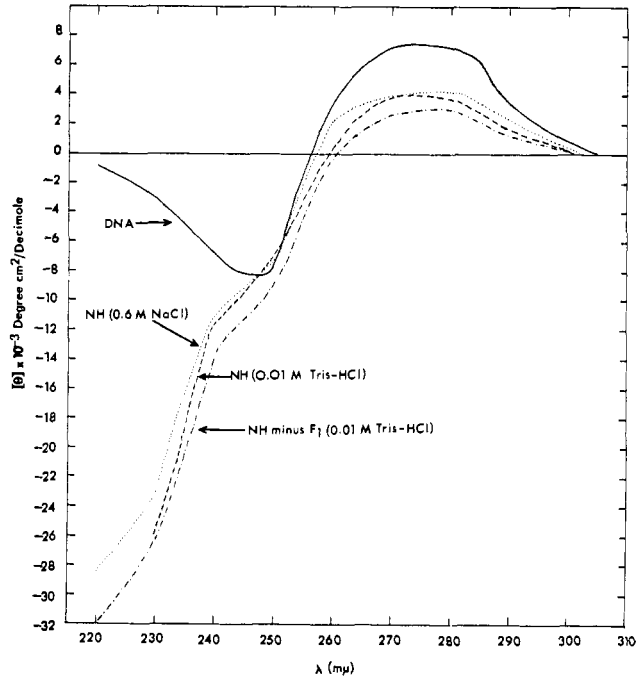

FIGURE 6: The circular dichroism of nucleohistone in 0.6 M sodium chloride–0.1 M Tris-HCl (pH 7.0) is compared to that of nucleohistone from which histone F_1 has been dissociated and subsequently dialyzed into 0.01 M Tris-HCl (pH 7.0). The CD of bovine thymus DNA (0.01 M Tris-HCl, pH 7.0) is also shown.

more nearly resembles that of DNA in the region 260–300 mμ. There is relatively little change in protein secondary structure as is evidenced by the constancy of the CD spectra in the range 220–250 mμ. It is interesting to note that the CD spectra of nucleohistone and of nucleohistone lacking F_1 in

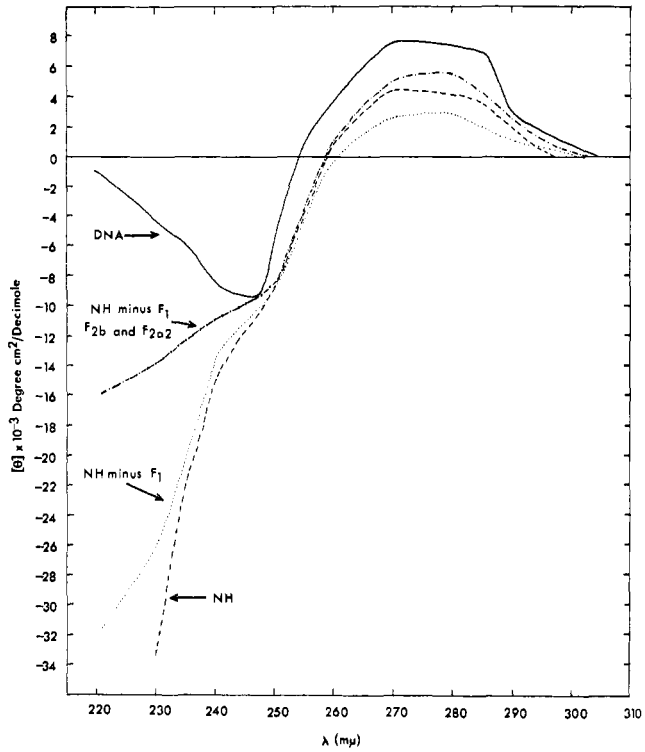

FIGURE 7: The circular dichroism of partially dissociated bovine thymus nucleohistone. Histone F_1 or histones F_1, F_{2b}, and F_{2a2} were selectively dissociated from nucleohistone as described previously (Bartley and Chalkley, 1972) and the residual nucleohistones subsequently dialyzed into 0.01 M Tris-HCl (pH 7.0). Bovine thymus DNA and nucleohistone (both in 0.01 M Tris-HCl, pH 7.0) are also shown.

0.01 M Tris-HCl are quite different in the range 260–300 mμ. Evidently at the lower ionic strength the nucleohistone lacking F_1 is particularly able to adopt the type of structure giving rise to low ellipticity in this spectral region, though this is clearly drastically modified if the ionic strength is raised to 0.6.

Circular Dichroism of Partially Dissociated Nucleohistone. We have recently described systems capable of selective removal of specific histones from DNA (Bartley and Chalkley, 1972). The effect of removing part of the histones from nucleohistone upon the circular dichroism of the residual nucleoprotein at an ionic strength of 0.01 is shown in Figure 7. As described above, removing F_1 actually decreases the positive band between 260 and 300 mμ and has little effect upon the CD spectra below 260 mμ even though approximately 25% of the histones have been dissociated. Presumably F_1 is making little contribution to the overall α-helix content of the nucleohistone. However dissociation of F_1, F_{2b}, and F_{2a2} increases the positive band (100%) and decreases the negative band (50%) so that the spectrum approaches that of DNA more nearly.

Circular Dichroism of Heat-Denatured Nucleohistone and DNA. If any part of the nucleohistone CD spectrum reflects the particular interactions of DNA and histone we expected that we might document it more clearly if we could destroy the conformation of native nucleohistone. Accordingly both nucleohistone and DNA were denatured by heating at 100° for 20 min and cooled in ice, and the CD spectra of the products were recorded. The data of Figure 8 show that the major effect of strand separation on free DNA is to decrease the 245 mμ ellipticity by some 2×10^3 (deg cm²)/dmol. Denaturation has little effect on the 275-mμ band. Upon denaturation the

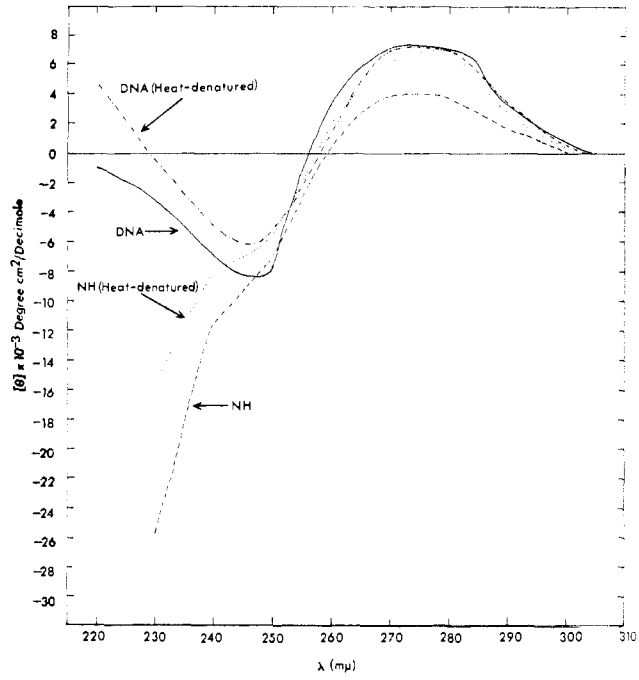

FIGURE 8: The effect of heat denaturation upon the circular dichroism of bovine thymus nucleohistone and DNA. Solutions of DNA or nucleohistone (0.01 M Tris-HCl, pH 7.0) were heated to 100° for 20 min, cooled in ice, and subsequently scanned.

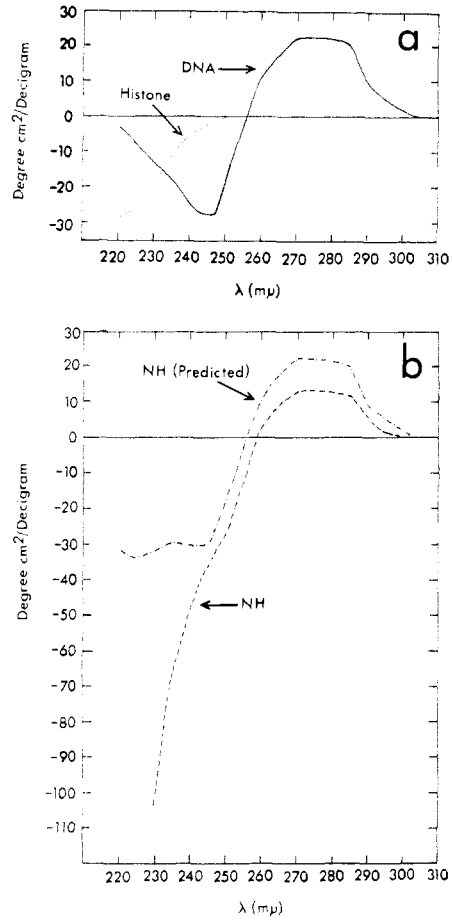

FIGURE 9: The predicted circular dichroism spectrum of bovine thymus nucleohistone in 0.01 M Tris-HCl (pH 7.0). (a) The CD spectra of histones and DNA (both in 0.01 M Tris-HCl, pH 7.0). (b) The predicted CD spectrum of nucleohistone from the addition of spectrum of histone to that of DNA is compared to the actual nucleohistone spectrum in 0.01 M Tris-HCl (pH 7.0).

230- to 260-mμ band of nucleohistone is decreased by 2–3 × 10^3 (deg cm²)/dmol, presumably reflecting the changes in the DNA contribution to the CD spectrum at these wavelengths. The most dramatic shift is seen in the 275-mμ band which has doubled upon denaturation so that it closely resembles that of single-stranded DNA. Apparently the result of strand separation in nucleohistone has been to destroy those factors contributing to the usually low CD band at 275 mμ while leaving unaffected the elements contributing to the spectrum in the 230- to 260-mμ region.

Construction of Circular Dichroism Spectra of Nucleohistone from Its Component Parts. The CD spectrum of native nucleohistone in 0.01 M Tris-HCl is different from that of a summation of the contributions of appropriate amounts of free DNA and free histone in 0.01 M Tris-HCl (pH 7.0) as is documented in Figure 9. In native nucleohistone there is a reduced positive ellipticity in the range 260–300 mμ and a much increased negative ellipticity in the range 220–250 mμ. The major criticism of using CD spectra of DNA and histone at low ionic strength (0.01) for construction of a composite spectrum is that when DNA and histone interact in the nucleohistone complex they each titrate a large amount of their mutual charge. We have therefore constructed composite curves from DNA and free histone in 2.0 M sodium chloride (Figure 10). It is immediately apparent that there is a reduction in the difference between the composite spectrum and that of native nucleohistone in the 260- to 300-mμ region, but that the bulk of the differences in the 220- to 250-mμ region remain. The circular dichroism spectrum of nucleohistone in the region 260–300 mμ can be ascribed to that of DNA alone as protein does not contribute to the spectrum at these wavelengths. The reduction in ellipticity of DNA in the nucleohistone complex in this region has been ascribed by Fasman *et al.* (1970) to the adoption of a supercoiled conformation. Increasing salt concentration has a similar (though smaller) effect (Figure 10) and this has been interpreted as a dehydration-induced shift in the tilt of the DNA bases (Li *et al.*, 1971). The observed decrease in nucleohistone ellipticity in the 260- to 300-mμ region could be due to a shift in the conformation of the DNA to the C form, either as a result of a histone-induced dehydration or because of the adoption of a different base tilting as a direct consequence of the DNA supercoiling. Perhaps the most likely interpretation is that both fractions contribute.

Discussion

We have described three experimental systems capable of dramatically modifying the hydrodynamic properties of nucleohistone, namely, urea solutions of intermediate concentrations, very low ionic strength and intermediate ionic strength (0.6 M NaCl). The first two systems maintain the histone complement and the final system removes histone F_1, though this is apparently not a major contributor to the hydrodynamic changes observed in 0.6 M NaCl as described in the Results section. The hydrodynamic changes we have observed are evidenced by substantial increases in the viscosity of the nucleohistone. Such changes could be due to a large increase in solvation or to an extension of the compact nucleohistone molecule. However we have calculated that the binding

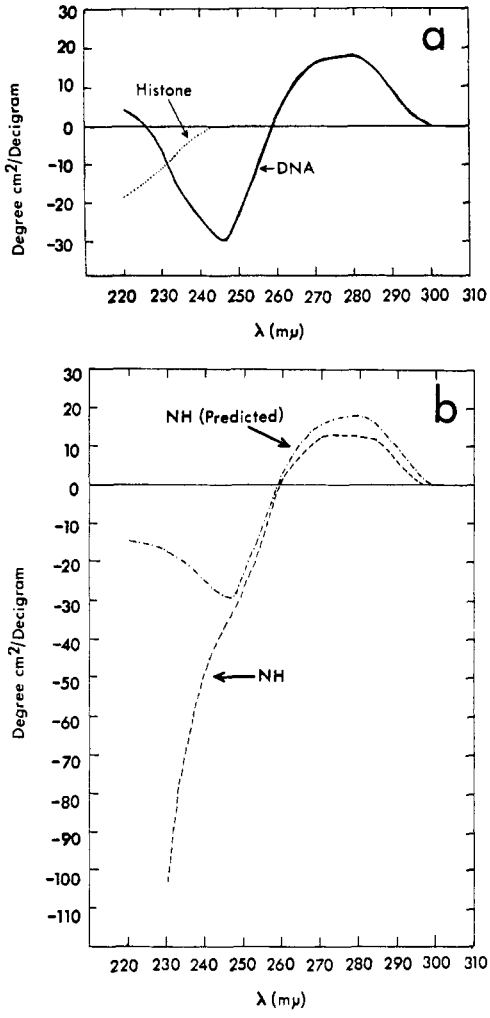

FIGURE 10: The predicted circular dichroism spectrum of bovine thymus nucleohistone from an addition of the spectrum of histone and that of DNA measured at high ionic strength. (a) The CD spectra of histones and DNA in 2.0 M NaCl–0.01 M Tris-HCl (pH 7.0). (b) The predicted CD spectrum of nucleohistone is compared to the actual nucleohistone spectrum in 0.01 M Tris-HCl (pH 7.0).

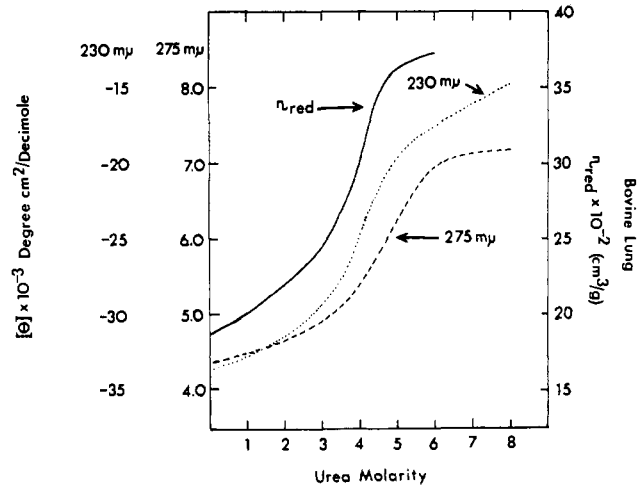

FIGURE 11: The dependence of the molar ellipticity and the reduced viscosity of nucleohistone on urea concentration. The molar ellipticity at 230 and 275 mμ of bovine thymus nucleohistone is compared to the reduced viscosity of bovine lung nucleohistone (118 μg/ml) at increasing urea concentrations.

to 1 g of nucleohistone by 1 g of solvent would lead to a 30% increase in viscosity (Bartley and Chalkley, 1968). The viscosity increases we are describing are almost an order of magnitude higher and it is unlikely that the massive hydration required is occurring, particularly as we have no evidence that this is so for the more highly charged DNA (Chattoraj and Bull, 1971). Accordingly, at this time we incline to the notion that in the systems described the nucleohistone molecules reflect in their hydrodynamic properties a partial extension of the supercoiled nucleoprotein molecules.

Urea causes the nucleohistone to develop a CD spectrum in the 260- to 300-mμ range which is essentially identical with that of DNA. The shift in the CD spectrum depends on the urea concentration as discussed below. The other two solvent systems which most likely do not disrupt protein secondary structure so dramatically also generate a shift toward that of DNA in the CD spectrum (260–300 mμ), but the spectrum never reaches that of DNA.

The effect of the different solvent systems upon the circular dichroism of nucleohistone in the 220- to 250-mμ range is also informative. Very low ionic strength and 0.6 M sodium chloride have almost no effect upon the negative ellipticity of nucleohistone. However increasing concentrations of urea generate a substantial decrease in ellipticity, probably related to a diruption of the histone secondary structure, though of course the histones remain bound to DNA by electrostatic bonds (Bartley and Chalkley, 1968). This observation is in agreement with our previous proposals that each of the three extending solvents works in a different manner, with urea functioning by destroying intramolecular hydrogen bonds or perhaps hydrophobic bonds, which may be involved in the maintenance of tertiary structure of the nucleohistone. If this notion is correct we might expect that the increase in viscosity and the decrease in ellipticity in the 230- to 250-mμ region should parallel one another as the urea concentration is varied. That this is indeed so is shown in Figure 11, in which figure we have also plotted the shift in positive ellipticity in the 260- to 300-mμ range. It is apparent that the latter shift likewise parallels the increase in viscosity at higher urea concentrations.

The data indicate that the dramatic decrease in ellipticity in the 220- to 260-mμ region is somehow related to the concomitant large increase in viscosity as the urea concentration increases, and to the shift towards ellipticity characteristic of DNA in the 260- to 300-mμ region. A reasonable interpretation of these observations is that the compact conformation of nucleohistone requires intact urea-sensitive histone interactions and that such a conformation does indeed modify the DNA circular dichroism in the 260- to 300-mμ region as has been suggested previously (Shih and Fasman, 1970). That urea disrupts the necessary histone interactions is indicated by the decrease in negative ellipticity in the 220- to 260-mμ region.

We have found it instructive to develop a model of the forces involved in nucleohistone structure based upon our analysis of the hydrodynamic and optical data. The model is based upon the following observations and assumptions (the observations are referenced). (1) The DNA molecule within the nucleohistone complex is in a regularly supercoiled conformation, so that its effective length is much decreased (Bayley *et al.*, 1962; Ohba, 1966; Pardon *et al.*, 1967; Bartley and Chalkley, 1968). (2) DNA would prefer to be in an extended rodlike conformation (even when the bulk of its negative charge is neutralized—as for instance in high concentrations of sodium chloride, *i.e.*, energy has to be expended

in order to compel the DNA to adopt the more compact conformation). (3) At physiological pH, histones are firmly bound to DNA by a combination of electrostatic bonds and hydrophobic bonds (Bartley and Chalkley, 1972) (probably of high specificity because of the conservative nature of most histone primary sequences (DeLange *et al.*, 1969; Panyim *et al.*, 1971)). (4) Histones have substantial amounts of α-helical structure when complexed with DNA (Jirgensons and Hnilica, 1965). (5) The nucleohistone complex has a negative charge density which is about one-fourth that of DNA (Olivera, 1967). (6) This charge is relatively uniformly distributed along the backbone of the molecule. (7) Histone possesses a considerable ability to aggregate (self-associate) under physiological condition of pH and ionic strength (Edwards and Shooter, 1969; Boublik *et al.*, 1970). (8) Those forces necessary for the supercoil conformation can be disrupted at fairly low concentrations of urea (2–4 M), particularly at lower ionic strengths. We suspect that these forces are either histone–histone or histone–DNA interactions. (9) The nucleohistone complex can extend if the DNA–histone electrostatic bonds are disrupted in 0.6 M NaCl. (10) The complex can extend if electrostatic repulsion along the nucleohistone backbone becomes overwhelming (as in low ionic strength).

The model for the structure of nucleohistone envisages the following. (1) The major destabilizing force on the compact form of nucleohistone structure is due to the rigidity of the DNA double helix and its tendency to revert to the rodlike form, and also to the negative charges along the nucleohistone backbone which tend to repel one another. (2) The major stabilizing force maintaining the supercoil is due to a torque generating a twisting of the DNA molecule. Such a strain can be relieved if the flexible rod collapses to a coiled structure. Topographical theory predicts that if a flexible rodlike element is twisted about the long axis through $n \times 360°$ (where n is any number or a fraction of a number) that n supercoils are generated in the relief of the twisting, provided that ends of the flexible rod are free to move in the direction of the long axis of the original rod (Vinograd *et al.*, 1968). (3) The torque about the long axis of the DNA molecule requires intact DNA–histone electrostatic bonds and intact urea-sensitive bonds. (4) The torque is generated in the following manner. Let us assume that the distances between histone–DNA electrostatic bonds are such that the histones may adopt the most favorable α-helical structure and the optimal stabilization of DNA–histone nonelectrostatic interactions only when nucleohistone is in the compact form. The electrostatic interactions serve to anchor the histones to the DNA, whereas the formation of secondary structure in histones and the subsequent interactions between histones and DNA serve to pull the anchored sites toward one another, thus generating the torque upon the molecule. A necessary corollary to this idea is that if nucleohistone were to be extended with the ionic interactions intact, the other histone–histone–DNA interactions should be displaced slightly from their thermodynamically most stable positions. The attempt to regain the most stable position provides the force for generation of the torque, and we may think of the histone α-helix structure acting as a spring. (5) That ultimately the stabilizing forces for the supercoiled conformatin come from histone intramolecular hydrogen bonds or histone–DNA hydrophobic bonds, with possible contributions from histone–histone intermolecular interactions.

In summary the supercoiling of nucleohistone arises from the balance of several forces which are produced when histones are anchored upon DNA; the overall resultant force generates a strain about the long axis of the DNA molecule which is relieved by the DNA adopting a more compact conformation.

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