Conformational Changes Associated with f-1 Histone–Deoxyribonucleic Acid Complexes. Circular Dichroism Studies*

G. D. Fasman, B. Schaffhausen, L. Goldsmith, and A. Adler

ABSTRACT: Mixtures of calf thymus DNA and the lysine-rich histone fraction, f-1, from the same tissue were examined under various conditions by means of circular dichroism. These DNA–histone complexes, which are a model system for chromatin, display conformational changes dependent upon solvent and upon complex composition and concentration. At moderate salt concentration (0.14 M NaF) the conservative circular dichroic spectrum of DNA becomes progressively distorted as increasing amounts of histone are added: the positive ellipticity band at 275 mμ decreases, the negative band at 245 mμ increases in magnitude, both bands are red shifted, and other alterations occur at lower wavelengths. These ellipticity changes are augmented by increases in complex concentration. At low ionic strength (0.01 M) the circular dichroic spectra of the complexes (known to be formed) are identical with that of the sum of free DNA and histone. On the other hand, small amounts of organic solvents increase the ellipticity changes. Conformational changes are not apparent upon binding of heat-denatured DNA to f-1 histone. The results are interpreted in terms of association of deoxyribonucleic acid–histone complexes and in terms of deoxyribonucleic acid hydration.

In contrast to the rapid progress made in studying the genetic systems of bacteria and phages, advances in understanding the genetic mechanisms of eucaryotes have been modest. This is partially due to the failure of understanding the role that the proteins play in nucleoproteins. Such proteins have been suggested to have both structural and regulatory importance (Bonner and Ts'o, 1964; Littau *et al.*, 1965). Chemical studies alone of these molecules have not, however, yielded an understanding of their dynamic biological mode of action.

The structure of the chromatin complex has been approached basically from three directions. The first employs model compounds such as poly-L-lysine and studies their interactions with DNA or synthetic polynucleotides (Leng and Felsenfeld, 1966; Tsuboi, 1967; Olins *et al.*, 1968; Davidson and Fasman, 1969; Shapiro *et al.*, 1969); the second utilizes isolated complexes of nucleohistone or chromatin (Bradbury *et al.*, 1962; Oriel, 1966; Bartley and Chalkley, 1968); and the third method involves examination of reassociated complexes of DNA and histones or residual complexes from which some component has been removed (Akinrimisi *et al.*, 1965; Johns and Forrester, 1969; Olins, 1969; Tuan and Bonner, 1969). Optical rotatory dispersion studies (Oriel, 1966; Tuan and Bonner, 1969) have shown that the rotatory properties of DNA are altered in nucleohistone, probably indicating a change in conformation of the DNA.

The lysine-rich fraction, f-1 of calf thymus histone, has been suggested to be involved in the maintenance of chromosome conformation (Mirsky *et al.*, 1968). For this reason, this fraction has been chosen for study of its interaction with calf thymus DNA. Circular dichroism has been utilized to examine these interactions, as the ellipticity bands of the DNA have no or modest contribution from the histone in the long-wavelength region of the DNA spectrum.

Experimental Section

Materials. The f-1 histone fraction was isolated from calf thymus tissue by the method of Kinkade and Cole (1966a). The thymus was obtained immediately after the death of the animal and frozen on Dry Ice. The f-1 migrated as one band on polyacrylamide gel as carried out by the method of Reisfield *et al.* (1962). The histone so obtained is reported to have a molecular weight of about 22,000; it has a high proline and alanine content, as well as about 25 mole % lysine (Teller *et al.*, 1965; Kinkade and Cole, 1966a). Protein concentration was determined by the microbiuret method using poly-L-lysine as a standard (Zamenhof and Chargaff, 1957).

As different batches of commercial DNA did not yield reproducible circular dichroic spectra, DNA was isolated from thymus tissue by a combination of high-salt, phenol, and chloroform–isoamyl alcohol extractions. The procedure of Zamenhof utilizing 0.1 M EDTA, pH 7.35, was used to obtain a high salt extract which was then precipitated with 95% ethanol (Zamenhof, 1957). The DNA was redissolved and treated with RNase using the method of Marmur (1961). The solution was then extracted three times with water-saturated phenol and spooled from ethanol. The resulting DNA was then purified according to the chloroform–isoamyl alcohol procedure of Marmur (1961) omitting the RNase, and the final stock solution was prepared in 0.14 M NaF at pH 7.0 and stored at 4°. The resulting DNA showed less than 0.5 % protein contamination according to amino acid analysis carried out on a Beckman amino acid analyzer by the standard method

* Publication No. 717 from the Graduate Department of Biochemistry, Brandeis University, Waltham, Massachusetts 02154. *Received February 26, 1970.* Presented in part at the 14th Annual Meeting of the Biophysical Society, Feb 25–27, 1970, Abstracts 10, p 107a. This work was generously supported by research grants from the National Science Foundation (GB-8642), the National Institutes of Health of the U. S. Public Health Service (GM 17533-09) and the American Heart Association (69-739). One of us (B. S.) is a National Science Foundation Predoctoral Fellow and (L. G.) is supported by a National Institutes of Health Biochemistry Training Grant (CA-5174-07), and is a Carl Herzog Fellow of the American Dermatology Association.

(Spackman *et al.*, 1958). There was no RNA detectable by the orcinol reaction (Schneider, 1957). The DNA showed a 42% hyperchromicity (OD_{260}) upon heating to 93°. DNA and DNA complexes were assayed primarily with the diphenylamine reaction (Ashwell, 1957), and the phosphate analysis of Ames and Dubin (1960) was also used. At low concentrations of f-1:DNA (10^{-4} M phosphate) the concentration of DNA was obtained by hydrolysis in 0.5 N perchloric acid (Ogur and Rosen, 1958) and by then using ϵ_{260} (molar PO_4) = 10.1×10^3 for the resulting mixture of mononucleotides. The molar extinction coefficient of complexes estimated from the perchloric acid method yielded a value of ϵ_{260} (mole phosphate) = 6.8×10^3. DNA was denatured by boiling for 20 min followed by quick cooling.

Solutions. Complexes were made by dialyzing from a solution at high ionic strength (2.0 M KF) down to the desired ionic strength in NaF in the manner described by Huang *et al.* (1964). Protein to DNA ratios (r) are reported as moles of amino acid residue per mole of nucleic acid phosphate. Unless otherwise noted, the solvent used was 0.14 M sodium fluoride at pH 7.0. Controls were run in sodium perchlorate and potassium chloride to eliminate the possibility of a specific ion effect.

Methods. The circular dichroism experiments were carried out on the Cary Model 60 recording spectropolarimeter with a Model 6001 circular dichroism attachment. All experiments, unless otherwise mentioned, were carried out at 23° in fused quartz, 5-cm, 1-cm, or 1-mm cells using an OD_{260} of no higher than 0.7, in the manner previously described (Adler *et al.*, 1968). Concentrations of DNA used were in the 10^{-5} to 10^{-3} M base range. Mean residue ellipticity, $[\theta]$, is reported in deg-cm^2 per dmole of nucleotide residue for DNA complexes. $[\theta]$ is based upon amide residue concentration in the case of pure histone.

The solutions showed slight opalescence, which increased at higher DNA and salt concentrations. Light scattering was observed in the absorption spectra of these latter solutions. However, the large circular dichroism changes observed could not be attributed entirely to such effects. The pH was measured with a Radiometer 25 pH meter with a combination electrode type GK 2021B. Ultraviolet spectroscopy was carried out on a Cary 14.

Results

Effect of Varying f-1:DNA Ratio. The circular dichroism spectrum of f-1 histone at pH 7.0, 0.14 M NaF, is shown in Figure 1. Above 250 mμ the spectrum is featureless; below 250 mμ the spectrum shows a negative band at 199 mμ, with $[\theta]_{199}$ −18,900. The spectrum has a small, but discernible shoulder at longer wavelengths. Such a spectrum corresponds to a random polypeptide conformation, although $[\theta]_{199}$ is smaller than that associated with 100% random coil (Greenfield and Fasman, 1969), in agreement with the results of other workers (Jirgensons, 1966; Bradbury *et al.*, 1967). The spectrum is independent of pH between pH 5.0 and pH 9.0. The circular dichroism spectrum of DNA is also shown in Figure 1. It exhibits the conservative spectrum typical of DNA. There is a positive ellipticity band at 275 mμ with $[\theta]_{275}$ 8500 and a negative band at 245.5 mμ with $[\theta]_{245.5}$ of −8850; the crossover point is at 256.5 mμ. At lower wavelength there is another positive band at 220 mμ with $[\theta]_{220}$ 3800 and another

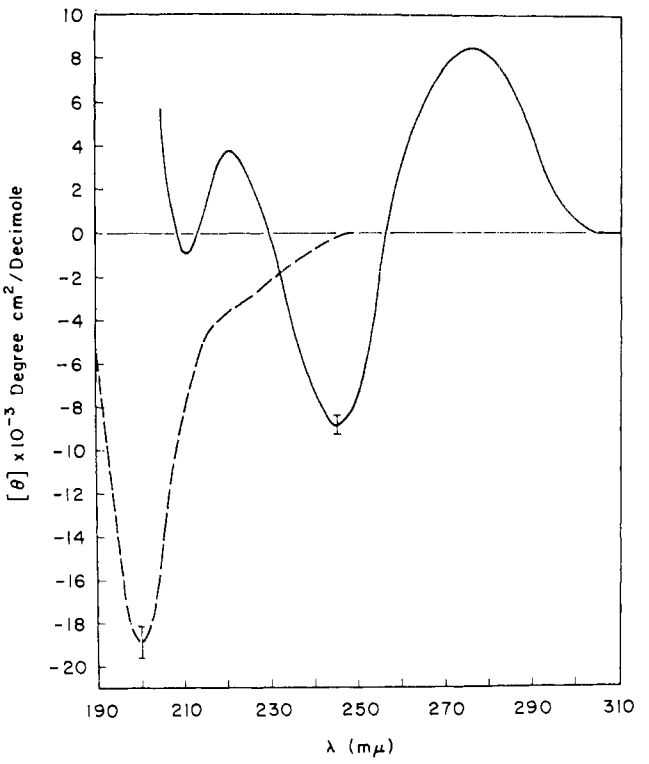

FIGURE 1: Circular dichroism spectra of calf thymus DNA and f-1 histone: (—) DNA, 1.2×10^{-3} M residue; (----) f-1 histone, 1.18×10^{-3} M residue. Solutions in 0.14 M NaF, pH 7.0. 1-mm cells were used.

negative band at 210 with $[\theta]_{210}$ of −1000. These values are in substantial agreement with the results of other workers (Brahms and Mommaerts, 1964; Sarkar *et al.*, 1967), although the values are slightly larger than previously reported. It has been observed, however, that particularly the low-wavelength region of the spectrum varied with the batch of DNA, at least in commercial preparations, probably indicating varying degrees of denaturation or protein content. It is clear from Figure 1 that there is relatively little overlap between the protein and nucleic acid spectra above 250 mμ, allowing the monitoring of changes in the DNA conformation at longer wavelength without having interference from the protein.

Complexing f-1 histone to DNA (10^{-3} M phosphate) in 0.14 M NaF affects the spectrum in several ways (Figure 2). As increasing amounts of histone are added, the peak at 275 mμ first decreases and red shifts until at a protein (mole of residue)/DNA(mole of phosphate) ratio of 1 (r = 1) it disappears. The negative ellipticity band at 245 mμ increases in magnitude with increasing ratios and shifts to 251 mμ at r = 1. The spectrum at low wavelength becomes more negative, so that even when corrected for the contribution of the f-1 histone, the former positive peak at 220 mμ now is a negative maximum between two negative bands. At r = 1 a shoulder is seen at 265 mμ on the negative band with maximum at 251, $[\theta]_{251}$ -16×10^3; an inflection point is found at 228 mμ and a large negative band is seen at 210 mμ, $[\theta]_{210}$ -23×10^3. Increasing the histone to DNA ratio decreases the net negative charge on the complex, and allows formation of associated complexes, or specific aggregates. It is these aggregates which create circular dichroism spectra greatly different from that of DNA,

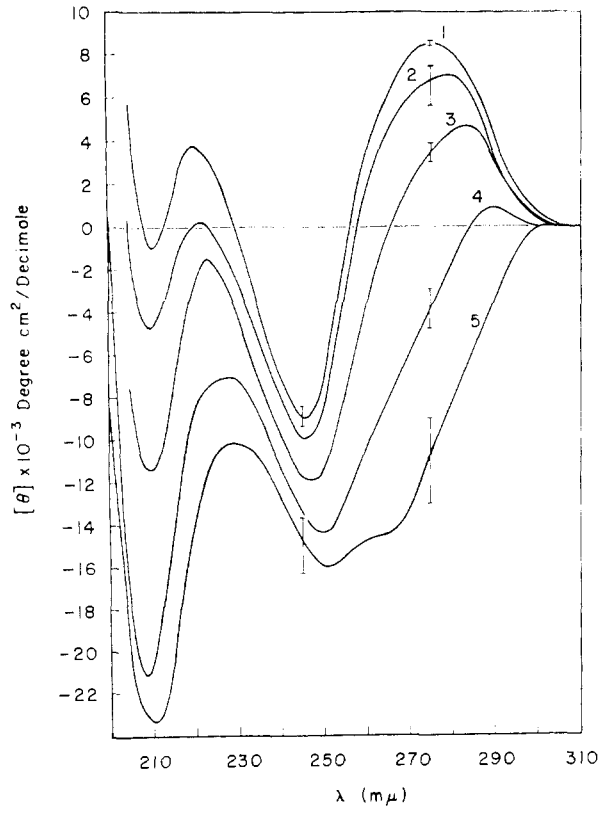

FIGURE 2: Circular dichroism spectra of f-1–DNA complexes as a function of r, the histone (mole residue):DNA (mole phosphate) ratio at 10^{-3} M DNA (mole phosphate), in 0.14 M NaF, pH 7.0: curve 1, native DNA; 2, $r = 0.25$; 3, $r = 0.50$; 4, $r = 0.75$; and 5, $r = 1.0$. Error bars represent reproducibility and noise dependence. Uncorrected for histone contribution. Path length, 1 mm.

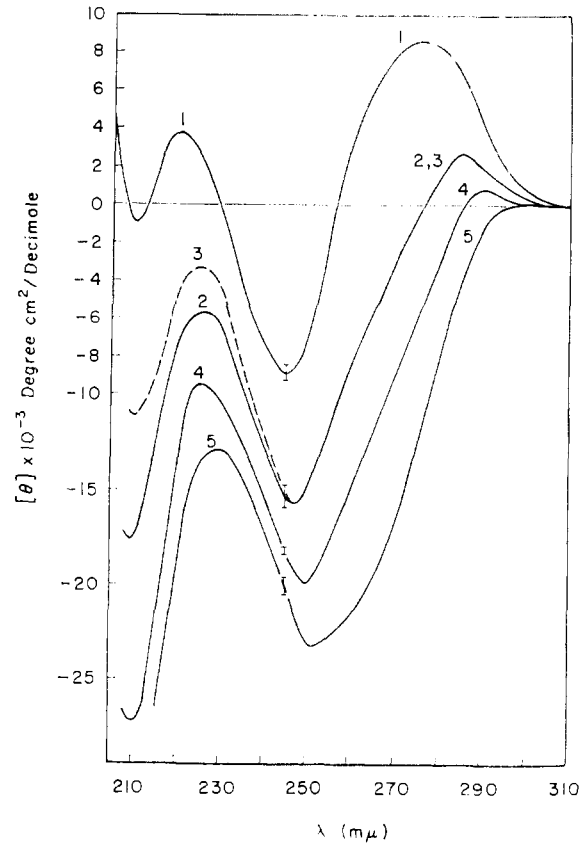

FIGURE 3: Circular dichroism spectra of f-1-DNA complexes as a function of r, the histone (mole residue):DNA (mole phosphate) ratio at 10^{-4} M DNA (mole phosphate, in 0.14 M NaF, pH 7.0: curve 1, native DNA; 2, $r = 0.75$; 3, $r = 0.75$, corrected for histone contribution; 4, $r = 1.0$; 5, $r = 1.25$. Path length varying from 1-cm to 5-cm cell.

as will become apparent later. At $r = 1$ the charge on the DNA is 28% neutralized.

Effect of Varying f-1–DNA Concentration. The observed changes in the circular dichroism spectrum of DNA upon addition of f-1 were found also to be dependent on the original concentration of DNA. In Figure 3 is seen the effect on the circular dichroism upon adding f-1 to a solution of DNA at 10^{-4} M (phosphate) in 0.14 M NaF, pH 7.0. The circular dichroism of DNA alone is not altered upon dilution. At 10^{-4} M complex concentration it can be seen that higher ratios of f-1:DNA are required to reduce the circular dichroism curves to comparable values found at 10^{-3} M complex. A ratio of 1.25 is required to reduce the $[\theta]_{275}$ to zero at 10^{-4} M DNA. However, when this band is reduced to zero, the band at 245 mμ, which shifts to 251 mμ, has become more negative than is found at 10^{-3} M. To illustrate the circular dichroism correction which may be applied for the histone contribution, in Figure 3, curve 3 is the corrected spectrum of curve 2 for $r = 0.75$. The correction changes values only below 250 mμ. Such corrections may not be precise due to changes in the histone conformation upon being complexed with the DNA. This cannot, however, be the primary explanation of the observed circular dichroism changes because a greater change is seen at 210 than at 220 mμ, which would not be expected for a coil–helix or a coil–β transition (Greenfield and Fasman, 1969). Further studies were carried out to investigate the effect of f-1:DNA concentration on the circular dichroism spectra. The results are shown in Figure 4 for an f-1–DNA complex of $r = 0.75$ in 0.14 M NaF, where the concentration of the complex is varied from 10^{-3} to 10^{-5} M (phosphate) DNA. Two plots are shown: (a) the ratio $[\theta]_{285}:[\theta]_{247}$ (where peak values were used), as well as (b) $[\theta]_{\sim 285}$, (at peak) as functions of complex concentration. It can be seen at 10^{-5} M DNA the values measured are approaching the value found for native DNA under similar conditions. However, as the complex concentration is increased, between 5×10^{-5} M and 4×10^{-4} M DNA there is a large decrease in $[\theta]_{285}$, and a similar decrease in the $[\theta]_{285}:[\theta]_{247}$ ratio. There after, at $r = 0.75$ it appears that increased concentrations of complex do not cause further large changes in the circular dichroism spectra. Thus, it appears that the interaction between complexes plays an important role in determining the final conformations. That is, formation of specific aggregates or super complexes is required for large changes in circular dichroism.

Effect of Ionic Strength. The results presented above appear to disagree with those of Olins (1969) which show no change in the conformation of DNA upon being complexed with f-1 histone, and the results of Tuan and Bonner (1969) with partially dissociated nucleohistones. The reason for the discrepancy between the Olins (1969) report and the present data are the differences in the ionic strength at which the experiments

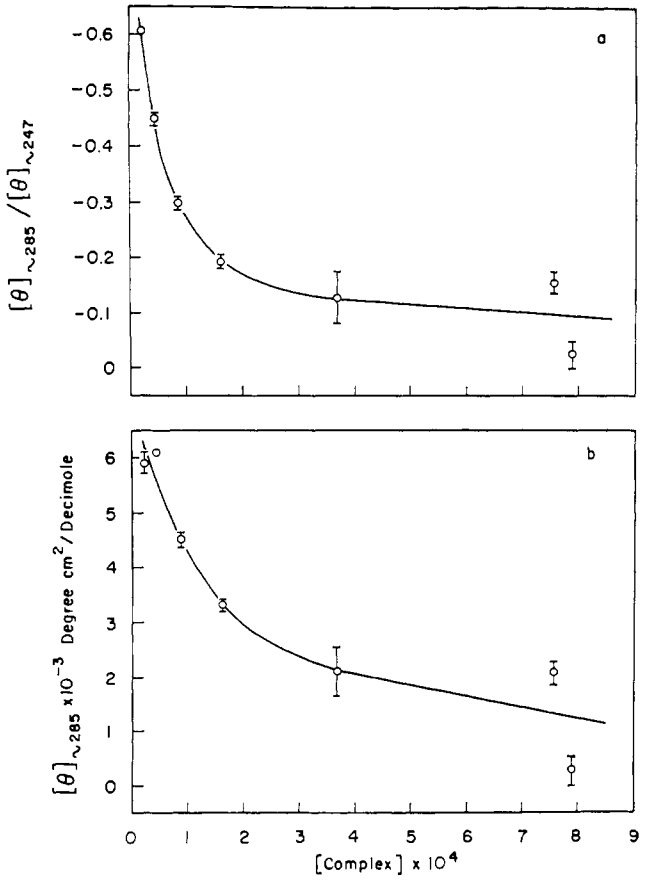

FIGURE 4: The dependence of the circular dichroism spectra upon f-1–DNA complex concentration: f-1–DNA at r = 0.75 in 0.14 M NaF at pH 7.0. (a) $[\theta]_{\sim 285}$:$[\theta]_{\sim 247}$ (peak values ratio) as a function of DNA concentration (M phosphate), termed complex concentration; (b) $[\theta]_{\sim 285}$ (ellipticity at peak) *vs.* complex concentration. Below 10^{-4} M, path length 5 cm; 10^{-4} M, 1 cm; 10^{-3} M, 1-mm cell used.

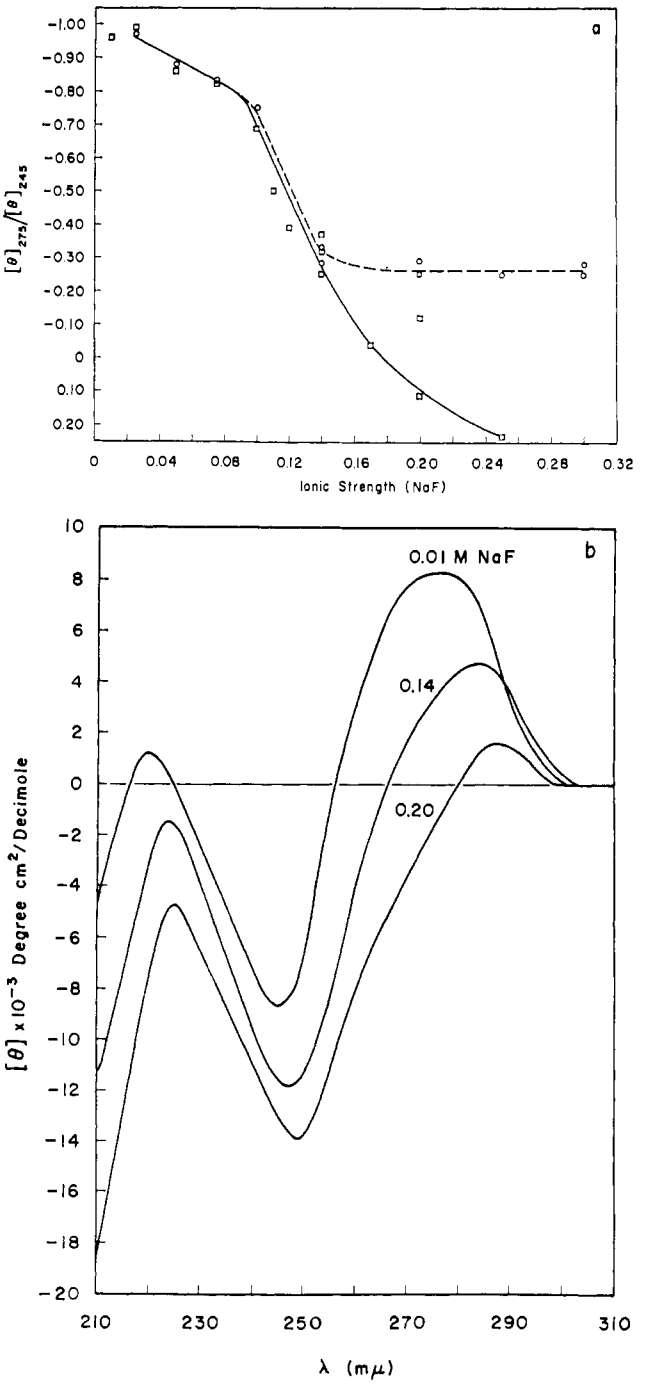

FIGURE 5: Circular dichroism. (a) The dependence of the circular dichroism ratio $[\theta]_{275}$:$[\theta]_{245}$ for f-1–DNA complexes (r = 0.5) as a function of ionic strength: salt NaF, pH 7.0; (⊙)10^{-4} M DNA (mole phosphate) measured in 1-cm cell; (□) 10^{-3} M DNA (mole phosphate) measured in 1-mm cell. (b) Circular dichroism spectra of f-1–DNA complexes as a function of ionic strength (NaF concentration) for 10^{-3} M (phosphate) DNA at r = 0.5; 1-mm path length.

have been carried out.[1] It was confirmed that at 0.01 ionic strength the circular dichroism spectra of f-1–DNA complexes are essentially those of native DNA, as reported previously (Olins, 1969). However, as the ionic strength is increased, the changes in the spectra become more and more pronounced. Binding at low ionic strength has been demonstrated by means of equilibrium dialysis (Akinrimisi *et al.*, 1965) and by the elevated and biphasic melting profiles of complexes (Huang *et al.*, 1964; Olins, 1969). Complexing at 0.01 M NaF was further demonstrated by filtering f-1–DNA complexes (0.5 ratio) through 0.45 μ Millipore filters (01300). Uncomplexed DNA quantitatively came through the filter while the complexes were retained.

The effect of variation in the ionic strength upon the circular dichroism of the f-1–DNA complexes at r = 0.5 is seen in Figure 5a. The change in circular dichroism is illustrated by the ratio $[\theta]_{275}$:$[\theta]_{245}$ and the dependence is shown for two concentrations of DNA, 10^{-3} M (mole phosphate) and 10^{-4} M.

[1] The present results cannot be compared to those of Tuan and Bonner (1969) in any simple manner, as pointed out by a referee. These authors found that removing the f-1 histone fraction from nucleohistone did not alter the DNA conformation, whereas the data herein show that adding f-1 to DNA can alter it. The possibility exists that f-1 may be responsible for altering the DNA conformation in forming a complex initially, but that, once altered, the other histones can maintain this conformation even when f-1 is removed. A similar statement may be applied to the recent finding (Bradbury and Crane-Robinson, 1970) that removal of f-1 does not affect the X-ray spacings of nucleohistone.

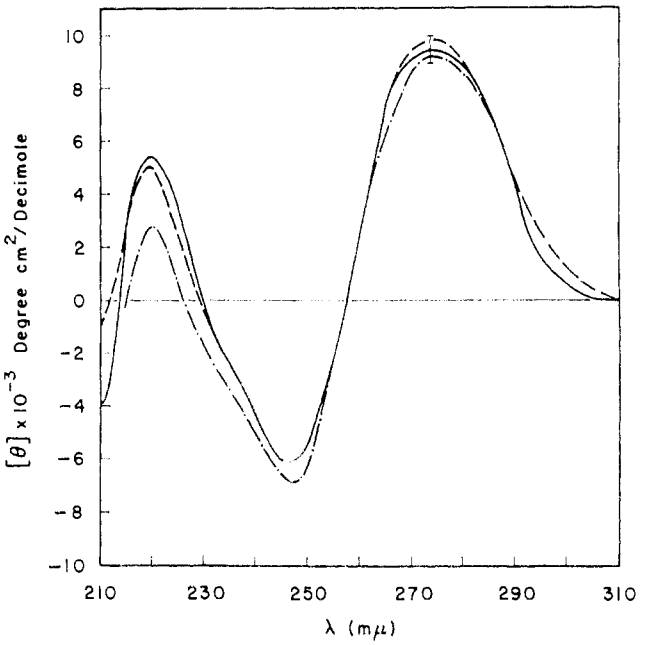

FIGURE 6: Circular dichroism spectra of f-1-denatured DNA complexes: denatured DNA(--); f-1-DNA, r = 0.5 (----); r = 1.0 (-·-·-). DNA concentration 10^{-4} M (phosphate), in 0.14 M NaF, pH 7.0. Path length, 1-cm cells. Data are not corrected for histone contribution.

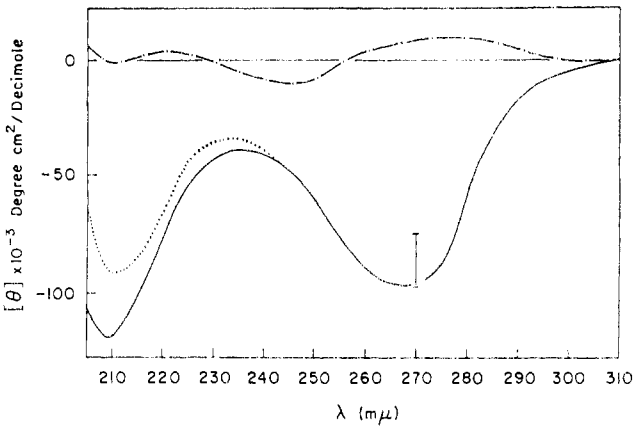

FIGURE 7: Circular dichroism of f-1-DNA complex at r = 3.0, in 0.14 M NaF, pH 7.0: Native DNA (-·-); f-1-DNA (——); curve corrected by substraction of histone contribution for f-1-DNA(·····). DNA at 10^{-3} M (phosphate) measured in 1-mm cell; DNA in complex at 10^{-4} M (phosphate) measured in 1-cm cell. Error bar represents possible error in concentration due to light scattering.

In Figure 5b is seen the circular dichroism spectral changes for f-1-DNA, r = 0.5, at 10^{-3} M (phosphate) DNA at three salt concentrations (NaF = 0.01, 0.14, 0.20 M). The spectra at 0.01 ionic strength are the same as that for native DNA. There is a decrease in the $[\theta]_{275}:[\theta]_{245}$ ratio which is roughly sigmoidal as a function of ionic strength. As the salt concentration is increased (up to about 0.2 M), there is increased shielding of the negatively charged complex. Therefore, there is a tendency for the shielded complexes to associate and to form supercomplexes. This results in the observed circular dichroism changes.

This system, however, is quite complicated; at low complex concentration (10^{-4} M DNA) a minimum value for the $[\theta]_{275}:[\theta]_{245}$ ratio is reached before 0.14 ionic strength. At higher concentration (10^{-3} M DNA) the minimum is not reached before 0.25 M, and thereafter the system becomes even more complicated; at higher salt concentration a combination of effects occurs. Increasing the ionic strength causes further circular dichroism change along with simultaneous decreased binding of the positively charged histone to the negatively charged DNA, again due to increased shielding. The effect of increasing the ionic strength at a fixed f-1:DNA ratio is similar to that observed for increasing the ratio of f-1:DNA at a fixed ionic strength (Figures 2 and 3). It is evident that both ionic strength and the concentration of the DNA are important factors in determining the magnitude of the changes, as can be seen in Figure 5.

Other Considerations. The conformational changes observed pertain only to native DNA. In Figure 6 the circular dichroism curves for heat-denatured DNA and f-1-denatured DNA complexes at two ratios are seen, and little change is noted. The curve for denatured DNA (10^{-4} M phosphate) agrees with that of Brahms and Mommaerts (1964). The two complexes at r = 0.5 and 1.0 show insignificant changes compared to denatured DNA and are within experimental error at high wavelength. Apparently, the histone does not affect the conformation of denatured DNA. When the curves are corrected at 250 mμ and below for the contribution of the random coil histone, the complexes and denatured DNA yield identical curves. The unchanged circular dichroism spectra of denatured DNA complexes are not simply due to a change in the binding constant, because Akinrimisi *et al.* (1965) have shown that the binding constant for f-1-DNA to native and denatured DNA is about the same in this ionic strength range. An analogous situation was also found with poly A. At pH 7.0 where poly A is single stranded, f-1 has little effect on the conformation as determined by circular dichroism measurements. At pH 4.3, on the other hand, where poly A is double stranded (Holcomb and Tinoco, 1965), there is a pronounced effect on the conformation.

The f-1 fraction is about 20% by weight of the total histone (Johns, 1964; Butler *et al.*, 1968). This corresponds to an amino acid residue to nucleic acid phosphate ratio (r) of about 0.6 in the nucleus. If, however, complexes are made with greater and greater ratios, eventually a form is obtained whose circular dichroism spectrum has a large negative band at 270 mμ, a negative maximum at 230 mμ, and a second negative band at 210 mμ. Such a circular dichroism curve is shown in Figure 7 for a ratio of 3.0. The spectrum in Figure 7 corresponds to one observed recently by Shapiro *et al.* (1969) for poly-L-lysine-DNA complexes at a 1:1 ratio. The spectrum is highly unusual as the main ellipticity band at 275 mμ is inverted and the mean residue ellipticities are exceptionally large for DNA. A calculated curve is also shown containing the correction due to the contribution of the random coil histone. This reduces the magnitudes of the bands below 250 mμ very slightly. This solution of complex showed a high degree of light scattering. An error estimate made for possible scatter is shown in the figure.

Preliminary circular dichroism studies (Shih and Fasman, 1970) on chromatin (10^{-3} M, 0.14 M NaF) have shown that the DNA in chromatin has a circular dichroism spectrum which indicates a changed conformation relative to the native DNA.

The circular dichroism peak lies at 280 mμ (shifted to the red) and $[\theta]_{280}$ 5000, which is comparable with curve 3 of Figure 2. Thus, the conformational change induced is not as drastic as that found for high f-1 :DNA ratios.[2]

In the course of this work on f-1 and DNA it became clear that the complexes which were studied represent associated complexes. For example, they may be centrifuged out of solution with relatively low gravitational fields. Furthermore, progressive circular dichroism changes were found under conditions favoring aggregation (higher ratio, complex concentration, and ionic strength). It also appeared that there was cooperativity of binding of the histone to DNA. For example, one could spin out a stoichiometric amount of DNA as complex from the solution, or the complex could be filtered with an 0.45 μ HA Millipore filter.

However, the changes in circular dichroism are not simply the result of the small to moderate amounts of light scattering (OD_{400}/OD_{258} ranged from 0.01 to 0.06) which usually accompanied solutions of aggregated complexes. First, any precipitation caused by aggregation would be expected to reduce ellipticities by removing complex from the solution; since either spectrophotometric (corrected for scattering) or chemical determination of DNA gives the same concentration, this is not the case. Second, a simple scattering phenomenon might be expected to affect the circular dichroism (like the absorption spectrum) in the manner: θ scattering $= k\lambda^{-4}$. This expression is not followed for the circular dichroism spectra of associated complexes, which usually differ more from DNA in the 260- to 280-mμ region than at 220 to 240 mμ. For example, in Figure 3, $[\theta]_{275}$ for curve 3 (which has been corrected for the contribution of histone) is 7800 more negative than $[\theta]_{275}$ for DNA. If the λ^{-4} expression were obeyed, this difference in $[\theta]$ would be 18,200 at 220 mμ; instead it is observed to be only 7300. Third, Urry and Ji (1968) have calculated the manner in which dispersion distortion due to light-scattering affects the circular dichroism of particulate systems. This distortion causes damping and red shifts of circular dichroism bands, but no changes in crossover wavelengths or in the sign of the bands, such as are observed for f-1–DNA complexes. Fourth, a similar decrease and shift of the 275 mμ DNA band is seen in nonscattering solutions of chromatin. Thus, although light-scattering phenomena may well be distorting the circular dichroism spectra of aggregated complexes, the major qualitative changes in circular dichroism upon complex formation appear to be real, and therefore attributable to some change in DNA conformation upon aggregation of complexes. If, however, these aggregates form specific liquid-crystal-like complexes (which may be possible if f-1 histone serves as a specific cross-linking agent), the changes in circular dichroism could not be attributable to a conformational change of the DNA, but, rather to the lattice spacings of the aggregates.

The conformational changes so far described are seen to be dependent on concentration, *i.e.*, to be a function of associated complexes. To investigate the forces responsible for this association to form super complexes, the effect of organic solvents was measured. As hydrophobic forces may be involved in such association, such solvents may indicate whether these forces play a role. Intracomplex charge attractive forces (between DNA and histone) would be enhanced as would intercomplex repulsive forces between negatively charged complex molecules. The effect of dioxane or methanol on f-1–DNA complexes at 10^{-3} M (phosphate) DNA at $r = 0.5$ in 0.14 M NaF at pH 7.0 was investigated. Low concentrations (10%) of methanol or dioxane (added to the original dialysis solvent at high salt) produced complexes with circular dichroism spectra having unusually low values at 245 mμ and having a small shifted peak at 288 mμ. In 10% dioxane $[\theta]_{288}$ 623 and $[\theta]_{249}$ −11,000, and in 10% methanol $[\theta]_{288}$ 677 and $[\theta]_{249}$ −11,900. These curves look similar to that found for the complex at 10^{-3} M and for a ratio $r = 0.75$ (Figure 2, curve 4). Thus the effect of the organic solvents was equivalent to increasing the f-1–DNA ratio, *i.e.*, it enhanced the effect of the histone present in the complex. If increasing the ionic strength (until about 0.2 M) resulted in greater shielding, and thereby greater intercomplex interaction (aggregation), then one might expect the organic solvents, with lower dielectric constant, to work in the opposite fashion and reduce the association of negatively charged complexes by increasing the charge repulsion. However, the opposite occurs; association apparently increases. Thus, these results would superficially appear to be contradictory. Obviously, the interaction is far more complex than simple electrostatic interactions, or hydrophobic interactions between complexes.

Two alternate explanations are possible: First, a lower dielectric constant might allow greater or tighter binding of f-1 to DNA. This, however, is contradicted by the melting curves of f-1–DNA in 50% methanol (Olins, 1969). Second, highly charged DNA (and f-1–DNA complexes) are not nearly as soluble in organic as in aqueous media, since nonpolar molecules cannot solvate DNA as well as water. In concentrations of dioxane or methanol too low to cause precipitation, the DNA (or complex) molecules form aggregates. This increased association (super complex formation) would, as shown previously, induce conformational changes in the DNA.

Discussion

The large circular dichroism changes accompanying the interaction of f-1–DNA can be attributed to a conformational change induced by the histone binding onto the double-strand DNA, although no or small ultraviolet absorption changes ensued. Numerous studies have established that changes in circular dichroism spectra can be associated with changes in the asymmetric architecture of the chromophores (Yang and Samejima, 1969). Langridge *et al.* (1960) have determined by X-ray diffraction the molecular configuration for DNA in the B form, the form which is thought to exist in aqueous media (Brahms and Mommaerts, 1964; Maestre and Tunis Schneider, 1969). In the B conformation the bases are stacked almost parallel to one another (5° angle of twist) and are almost perpendicular to the helical axis (2° tilt). In contrast to the results of the present paper, several X-ray diffraction studies have concluded that the DNA configuration found in nucleohistones remains in the B form (Wilkins *et al.*, 1959; Zubay and Wilkins, 1962, 1964; Pardon *et al.*, 1967); similar conclusions have been arrived at by solution studies (Bradbury *et al.*, 1962). Pardon *et al.* (1967) however have remarked that the DNA reflections in X-ray diffraction studies are poorly oriented, and have suggested that nucleohistones *might* be

[2] Since this paper was submitted two other circular dichroism studies on chromatin have appeared which agree with the results reported herein (Permogorov *et al.*, 1970; Simpson and Sober, 1970).

supercoiled, as was proposed by Zubay (1964). (See Bradbury and Crane-Robinson (1970) for a review of physical studies on nucleohistones.) Previous conformational studies on DNA interactions with model compounds or with salts or organic solvents are informative. The interaction of poly-L-lysine with DNA was shown to cause significant rotatory changes (Cohen and Kidson, 1968; Shapiro *et al.*, 1969). The circular dichroism spectrum was seen to lose its conservative nature, and a large negative band appeared at 274 mμ (Shapiro *et al.*, 1969) similar to that observed with f-1–DNA at $r = 3$. The circular dichroism spectrum of DNA in 95% ethylene glycol was also seen to lose its positive circular dichroism band with the appearance of an enlarged negative band at 250 mμ (Green and Mahler, 1968), while 80% ethanol caused an increase in the 275-mμ band and a decrease in the 245-mμ band (Brahms and Mommaerts, 1964), a change in spectrum from conservative to nonconservative. The latter spectrum is similar to that found for RNA, and perhaps typical of the A form of DNA (3% Na content), as produced at 66% relative humidity with DNA films (Maestre and Tunis Schneider, 1969). The effect of 4–6 M NaCl, or LiCl on DNA was to diminish the cotton effects of the optical rotatory dispersion spectra (Tunis and Hearst, 1968a) and reduce the circular dichroism 275-mμ band to zero (Maestre and Tunis Schneider, 1969). Similar circular dichroism spectra could be obtained at 66% relative humidity with the lithium salt of DNA and it was postulated that this is the spectrum of DNA in the "C" form (Maestre and Tunis Schneider, 1969). Thus the circular dichroism spectra of DNA can be used to monitor conformational changes.

Theoretical work of Tinoco (1964) has suggested that the conservative circular dichroism spectrum of DNA is the result of base stacking perpendicular to the helical axis. Furthermore, the nonconservative nature of the RNA spectrum, for example, is attributed to the large tilt of the bases with respect to the helical axis (Tinoco, 1968); more recent calculations support such a hypothesis (Johnson and Tinoco, 1969). The circular dichroism changes observed for f-1–DNA complexes may likewise be due to base tilting. Bush and Brahms (1967), on the other hand, have suggested that the nonconservative behavior is due to the presence of a band outside the range of measurement.

The major effects observed in the studies reported herein may be summarized as follows: (1) the dependence of the magnitude of circular dichroism changes upon the f-1 : DNA ratio; (2) the dependence of the magnitude of the circular dichroism changes upon the concentration of f-1–DNA; (3) the ionic strength dependence of the conformational change due to f-1–DNA complexes; and (4) the effect of organic solvents on the circular dichroism of the complexes.

The first observation can be explained on the basis of the higher the coverage of the DNA, the larger the induced change. Thus as the ratio continuously changed from zero to 0.6 (the ratio found for f-1 : DNA in native nucleohistone), a larger conformational change is induced. The circular dichroism spectrum of chromatin is very similar to that found for $r = 0.5$ at 10^{-3} M (Figure 2). However, it is seen that this amount of binding does not appear to be specific, for the induced change continues as higher ratios are produced. At a ratio of $r = 3$ (Figure 7) the circular dichroism curve has become completely inverted in the 270-mμ region, similar to that found for poly-L-lysine–DNA (Shapiro *et al.*, 1969). The obvious role of charge neutralization for binding of the histone to DNA is observed. As about 72% of the f-1 histone is nonbasic (Kinkade and Cole, 1966b) on a residue mole basis, one could theoretically bind about 4 times the residue molar amount of histone to DNA ($r = 4$) before total charge neutralization occurred. Thus the effect upon the circular dichroism of increasing the ratio up to 3, where a completely different circular dichroism pattern is found (Figure 7), is understandable in terms of attractive forces and binding.

However, the binding of histone to DNA is not a sufficient explanation. At low ionic strength or with denatured DNA the histones have been shown to bind (Akinrimisi *et al.*, 1965) without a corresponding change in the circular dichroism spectrum, so that binding is not the sole criterion for conformational change. However, the shielding of the residual negative charge on the complexes may be important if association (aggregation) of complexes plays an important role in the observed circular dichroism changes. The sharp dependence of $[\theta]_{275}$ *vs.* ionic strength (Figure 5) over the range of 0.01 to 0.14 M NaF illustrates the importance of such shielding, and explains why some previous investigators have failed to observe circular dichroism changes with complexes at low salt concentration (Olins, 1969), while others (Oriel, 1966; Tuan and Bonner, 1969) have observed altered DNA conformation in nucleohistone under different conditions. However, if shielding alone determined the extent of the interaction between complexes, then the effect of organic solvents would be expected to increase the charge repulsion, and also to weaken any hydrophobic interactions, thus reducing association of complexes and thereby reducing the effect of the histone on the DNA spectra. But the reverse is true and the organic solvent further amplifies the conformational effect on the DNA. Thus shielding alone, or change in dielectric constant, cannot be considered the only factor; solvation of complexes and its effect upon aggregation should also be considered. The other major consideration is the effect of the complex concentration on the magnitude of the circular dichroism changes. At low concentrations (10^{-5} M) the circular dichroism of the complex in 0.14 M NaF is approaching that found for native DNA. As the concentration is increased the circular dichroism changes increase until about 4×10^{-4} M. Thus another requirement for the observed conformational change *is the association of complexes*, perhaps to form supercoils as previously suggested (Cole, 1962). It should be emphasized that the aggregates of complexes responsible for the circular dichroism changes are not composed of f-1 DNA molecules attached to one another at random. On the contrary, the complexes associate in a highly specific manner, dependent upon concentration, ratio, and solvent conditions, to induce specific conformational changes in the DNA. These changes are reflected in the reproducible alterations in circular dichroism spectra. The concentration of nucleohistone in the nucleus is about 0.7 M (phosphate) (Stern and Mirsky, 1953), much higher than used in these studies, and the ionic strength is at least isotonic. Thus the packaging of the nucleohistone in the nucleus or the association of the histone–DNA complexes can cause considerable conformational change. Similar observations have been noted for the DNA in the interior of phage heads (Maestre and Tinoco, 1967).

The conformational changes associated with the f-1–DNA complex are influenced by a variety of factors: residue ratio, ionic strength, complex concentration, organic solvents, and

DNA denaturation. There seems to be little in common among these various factors except for the extent to which they influence specific aggregation of f-1–DNA complexes. However, if association to form super complexes is the only source of the circular dichroism changes, many points remain obscure: the effect of DNA denaturation is not explained, the role of organic solvents is obscure, and it is not clear how aggregation alone can induce a conformational change.

There is another consideration which may be of common concern to all the above factors, and that is their effect on the hydration of DNA. Numerous authors have discussed the degree of hydration of DNA and its implication for DNA structure (Wang, 1955; Falk *et al.*, 1962; Falk *et al.*, 1963a; Lubas and Wilczok, 1966; Gray *et al.*, 1967; Tunis and Hearst, 1968b). It is clear that bound water is necessary for the maintenance of DNA structure (Franklin and Goslin, 1953). The stability of the B form depends on the hydration of the bases which in turn plays the important role in determining conformation. As the hydration of the bases is altered, the helical DNA structure begins to break down (Falk *et al.*, 1963b). It thus appears possible that the change in state of hydration of DNA can also play an important role in the conformational effect occurring in the f-1–DNA interaction and between complexes.

Denatured DNA is apparently less hydrated than native DNA (Gordon *et al.*, 1965; Lubas and Wilczok, 1966; Tunis and Hearst, 1968b). If such changes in hydration can be accepted, then it is not unreasonable to attribute the lack of a conformational change upon complex formation of denatured DNA with f-1 as being due to this factor. Lysine-rich histone is apparently important in maintaining the cross-linked structure of nucleoprotein in interphase chromatin (Littau *et al.*, 1965) and in metaphase chromosomes (Mirsky *et al.*, 1968) the arginine-rich histone does not seem to be as important. This may be explicable in terms of hydration. Leng and Felsenfeld (1966) have shown that poly-L-lysine binds preferentially to (A + T)-rich regions in DNA. A + T pairs have been shown to bind about two more molecules of water than a G + C pair (Tunis and Hearst, 1968c). Perhaps the relative hydration plays an important role in this binding.

The conformation of DNA in f-1–DNA complexes at 0.14 M ionic strength is seen to be altered relative to native DNA in solution. The circular dichroism changes observed could be due to tilting of the bases with respect to the helical axis (Wilkins *et al.*, 1959; Zubay and Wilkins, 1962, 1964; Brahms and Mommaerts, 1964; Shapiro *et al.*, 1969; Yang and Samejima, 1969; Johnson and Tinoco, 1969). However, a difference in the dimensions of the helix could likewise cause similar changes. The dependence of the conformation on the f-1 : DNA ratio, concentration, ionic strength, and organic solvents is explicable partially in terms of their effects on the hydration of the DNA. Both specific aggregation of complexes and the addition of organic solvents would tend to reduce hydration of complexes, which in turn may determine how the f-1 histone can perturb the native structure. Hydration operates through altering the stability of the native structure, allowing the f-1 to induce change, and perhaps by affecting differences in hydrophobic binding between the amino acid side chains in the protein and the purine and pyrimidine bases of the DNA.

Coupled with these interactions is the effect of the association of the complexes, forming super complexes, causing pronounced changes in the circular dichroism spectra. Thus the packaging in the nucleus would be sufficient to cause conformational changes of the DNA. If the f-1 histone does play a role in the regulatory mechanism, perhaps it is due to the manner in which it controls the association of the DNA in the chromatin, bringing about the necessary conformational alteration blocking transcription. Such conformational changes may also be important in maintaining chromosomal structure in mitotic cells.

Acknowledgment

We thank Drs. E. M. Bradbury, H. Sorber, and R. Simpson for preprints of unpublished work, and Dr. W. B. Grazer for helpful discussions.

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