Circular Dichroism Studies of Deoxyribonucleic Acid Complexes with Arginine-Rich Histone IV (f2a1)*

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ABSTRACT: The conformation of calf thymus DNA complexes with a homogeneous calf thymus arginine-rich histone IV (f2a1) has been studied by means of circular dichroism. Complexes formed by annealing during NaCl–gradient dialysis in the presence of 5 M urea have characteristic circular dichroism properties dependent on the ionic strength at which urea is removed. Complexes formed by removal of urea at 0.15 M NaCl show changes in the circular dichroism spectra (in 0.14 M NaF) of the DNA. With increasing amounts of histone the 275-mμ ellipticity band increases in amplitude and blue shifts, the negative band at 245 mμ decreases, and enhancement of the negative band centered at 305 mμ occurs. The maximum change is observed at a histone:DNA ratio (r) of 1.5 amino acid residues to nucleotide residue. As r becomes larger than 1.5, the changes decrease. The nonconservative spectrum of the complex at $r = 1.5$ is very similar to that of double-stranded RNA or the theoretically calculated A form of DNA. This complex is very stable, shows no circular dichroism time effect for more than 2 weeks, and is not concentration dependent. The circular dichroism spectrum of native DNA can be restored by adding 0.1% (w/v) sodium dodecyl sulfate to the solution. Complexes formed by removal of urea at 0.015 M NaCl show no change in the circular dichroism spectrum of DNA measured in 0.01 M NaF. Since DNA–histone complexes are more soluble in 0.01 M than in 0.14 M NaF, the observed changes in conformation of the DNA appear to be dependent on the specific association state of the complex. The plasticity of DNA–histone complexes, allowing different specific association states, may have important implications in structural and functional changes of chromosomes particularly during cell division. The conformation of free histone IV is dependent on ionic strength; at 0.01 M NaF (pH 7.0) it is a random coil, while at 0.14 M NaF it becomes partially helical. The structure of the histone in complexes is dependent on its binding to DNA, rather than upon the ionic strength.

The genetic material of higher organisms appears to be much more complex than the genomes of viruses and bacteria, of which elegant molecular details have been elucidated in the past few decades. One aspect of this complexity is due to the interaction of DNA with histones, non-histone proteins, and chromosomal RNA in the eukaryotic chromosomes. For a fundamental understanding of transcriptional control and the mechanism of genome replication in the higher organisms, a detailed knowledge of the molecular structure and interaction of this nucleic acid–protein complex is essential.

Circular dichroism and optical rotatory dispersion have been shown to be potent tools to probe the structural features of macromolecules in solution. Studies on chromatin revealed that the conformation of DNA in the native complex is different from that of isolated DNA (Tuan and Bonner, 1969; Sponar *et al.*, 1970; Permogorov *et al.*, 1970; Simpson and Sober, 1970; Shih and Fasman, 1970). A simplified system in which molecular details of DNA–histone interaction can be obtained is DNA complexes with purified histone species. In this manner specificity relationships between histone and DNA dependent on amino acid and nucleotide composition and sequence may perhaps be elucidated. Under specific annealing conditions, DNA can form well-defined molecular complexes with purified histone fractions. This can be achieved by a decreasing salt-gradient dialysis; for histones other than lysine-rich f1 the presence of urea is needed to prevent aggregation of histones (Trautman and Crampton, 1959; Edwards and Shooter, 1969). Conformational alteration of DNA by complexing with f1 has been observed (Fasman *et al.*, 1970b).

In the present study, DNA complexes with arginine-rich histone IV (f2a1 in the nomenclature of Johns, Phillips, and Butler; see Fambrough and Bonner, 1969) have been studied by means of circular dichroism to investigate the conformational consequences of their interaction. This histone species is of special interest because of its homogeneity and known sequence (DeLange *et al.*, 1969a). Differences in the induced circular dichroism changes of DNA upon complexing with histone IV and with lysine-rich f1 have been observed.

Material and Methods

Arginine-rich histone IV was isolated from calf thymus. Whole histone was extracted with 0.4 N H_2SO_4 from crude chromatin (Shih and Bonner, 1969). It was then fractionated by Amberlite CG-50 (Mallinckrodt Chem. Co., New York) chromatography to obtain the arginine-rich histones (Bonner *et al.*, 1968). Histone IV was separated from III and other contaminants by Bio-Gel P-60 (Bio-Rad Lab., Calif.) chromatography (Fambrough and Bonner, 1969). Fractions containing IV were pooled from several successive runs and rechromatographed on Bio-Gel P-60. The elution profiles and experimental conditions are presented in Figure 1. The elution profile in Figure 1b, as analyzed by the DuPont 310 curve resolver, shows about 5% contamination. Histone IV thus purified moved as a single band on polyacrylamide gel electrophoresis (Bonner *et al.*, 1968) and possessed the same electrophoretic mobility as the histone IV band of whole histone gel.

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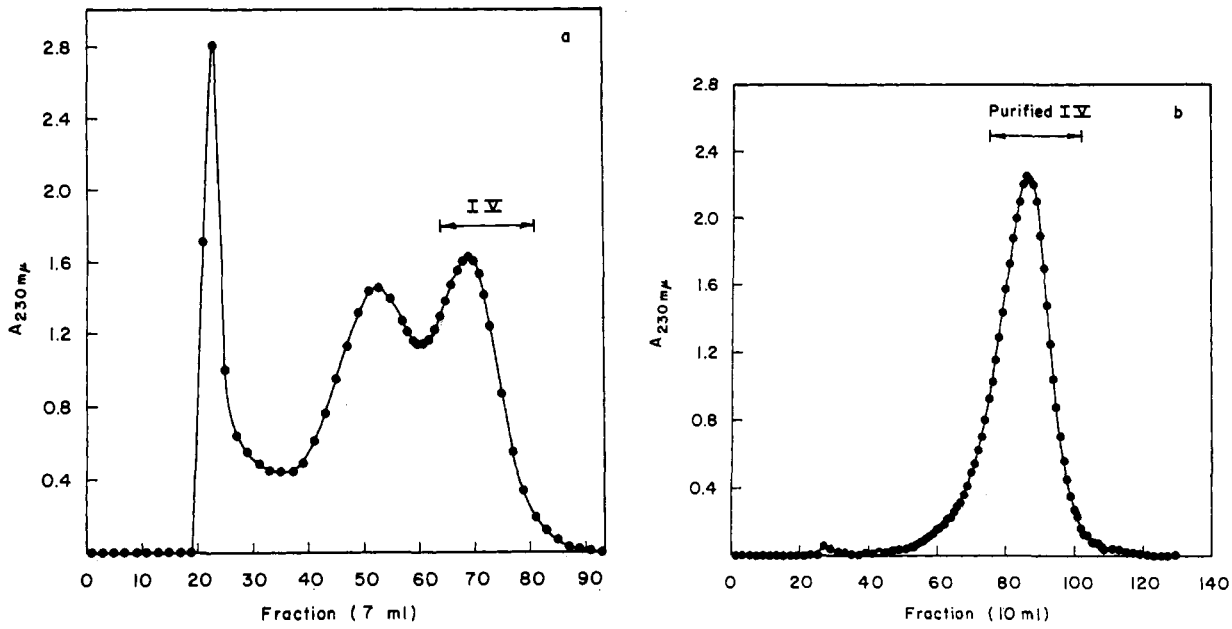

FIGURE 1: Purification of histone IV by Bio-Gel P-60 chromatography. (a) The arginine-rich histones III–IV from Amberlite CG-50 chromatography were dissolved in 8 M urea for 2 days in the cold. After brief dialysis against 0.01 N HCl, about 150 mg of histones was applied to a Bio-Gel column of the size 2.8 × 120 cm. The column was then eluted at the flow rate of 0.5 ml/min with 0.01 N HCl saturated with $CHCl_3$ at room temperature. (b) Fractions containing histone IV from (a) were pooled, condensed, and lyophilized. Histone IV (140 mg) was rechromatographed on a Bio-Gel column of the size 4 × 100 cm at the flow rate of 0.7 ml/min. The tailing was cut off, and purified histone IV was concentrated by ultrafiltration (Diaflo Membrane UM-2, Amicon Corp., Mass.) and lyophilized to yield its HCl salt.

Calf thymus DNA was purified by the same procedures used earlier (Fasman *et al.*, 1970b). The protein and RNA contents were, respectively, less than 0.3 and 0.2%.

Histone-DNA complexes of different ratios were prepared by a salt-gradient dialysis in the presence of 5 M urea at 4°C (Shih and Bonner, 1970). A step gradient of NaCl of 2, 0.9, 0.6, 0.3, and 0.15 M in 5 M urea was used. All steps were for 3 hr except overnight at 0.3 M NaCl. Urea was finally removed by dialysis against 0.15 M NaCl. Solvents were all buffered at pH 7.0 in 0.01 M Tris. Samples were then extensively dialyzed against 0.14 M NaF and 0.001 M Tris (pH 7.0) for circular dichroism measurements. This procedure will be referred to as *Dialysis A*. In some experiments, gradient dialyses were extended to 0.015 M NaCl, urea was the nremoved by dialysis against 0.015 M NaCl, and the solvent changed to 0.01 M NaF and 0.001 M Tris (pH 7.0) for circular dichroism measurements. This is procedure *Dialysis B*. As will be shown later, this procedure yielded complexes possessing completely different circular dichroism spectra from those prepared by removal of urea at 0.15 M NaCl.

To demonstrate that all the histone is bound in the complexes, prepared by dialysis B, the following two methods were employed. (1) A complex of $r = 1.5$ at 3.1×10^{-4} M (nucleotide) in 0.01 M NaF–1×10^{-3} M Tris (pH 7.0) was centrifuged at 130,000*g* for 21 hr (rotor temperature, 5°). There was no histone or DNA remaining in the supernatant as seen from the ultraviolet absorption spectra (360–220 mμ). The control experiments showed that all the DNA was sedimented and all the free histone remained in the supernatants under the same condition. (2) The same complex in the same solvent (at 1.5×10^{-3} M) was filtered through an Amicon Centrifluo ultrafiltration cone, CF 50A (Amicon Corp., Lexington, Mass.), which allows passage of molecules below mol wt 50,000. There was essentially no DNA or histone found in the filtrate; 31% of free histone alone was found to pass through the filter. For complexes prepared by dialysis A, these same experiments cannot readily be performed because histone IV is aggregated in 0.14 M NaF and cannot be completely separated from DNA and histone–DNA complexes. However, as 0.14 M NaF and/or urea is not able to dissociate histones from DNA, it is reasonable to expect that all the histone would be bound in the complexes formed by dialysis A.

A control DNA ($r = 0$) sample has been included in every procedure of complex preparation and manipulation. There is no change in its circular dichroism spectrum as compared to the original DNA stock solution. Although DNA heat stability is decreased in urea solutions (Shih and Bonner, 1970), in the presence of 0.015 M salt and at low temperature (4°), native DNA structure can be preserved.

All chemicals used were of analytical reagent grade obtained from commercial sources.

Circular dichroism spectra were measured with a Cary 60 recording spectropolarimeter with a Model 6001 CD attachment. Measurements were performed under a nitrogen atmosphere at 23° in fused quartz cells with optical path lengths ranging from 0.1 mm to 5.0 cm. OD_{260} of the DNA samples was about one unless otherwise specified. The machine was set for a slit program of 15 Å and appropriate time constant and scanning speed were selected to minimize noise. Mean residue ellipticity, $[\theta]$, is reported in (deg cm²)/dmole on the basis of DNA nucleotide concentration for complexes, and amino acid residues for histone.

Ultraviolet absorption spectra were carried out on a Cary 14 recording spectrophotometer at 23°. Some concentration determinations were performed on a Zeiss PM QII spectrophotometer.

DNA concentration in the complexes was determined by the diphenylamine reaction (Burton, 1956) or simply from OD_{260} of 0.5 N $HClO_4$ hydrolysates (10 min in boiling-water

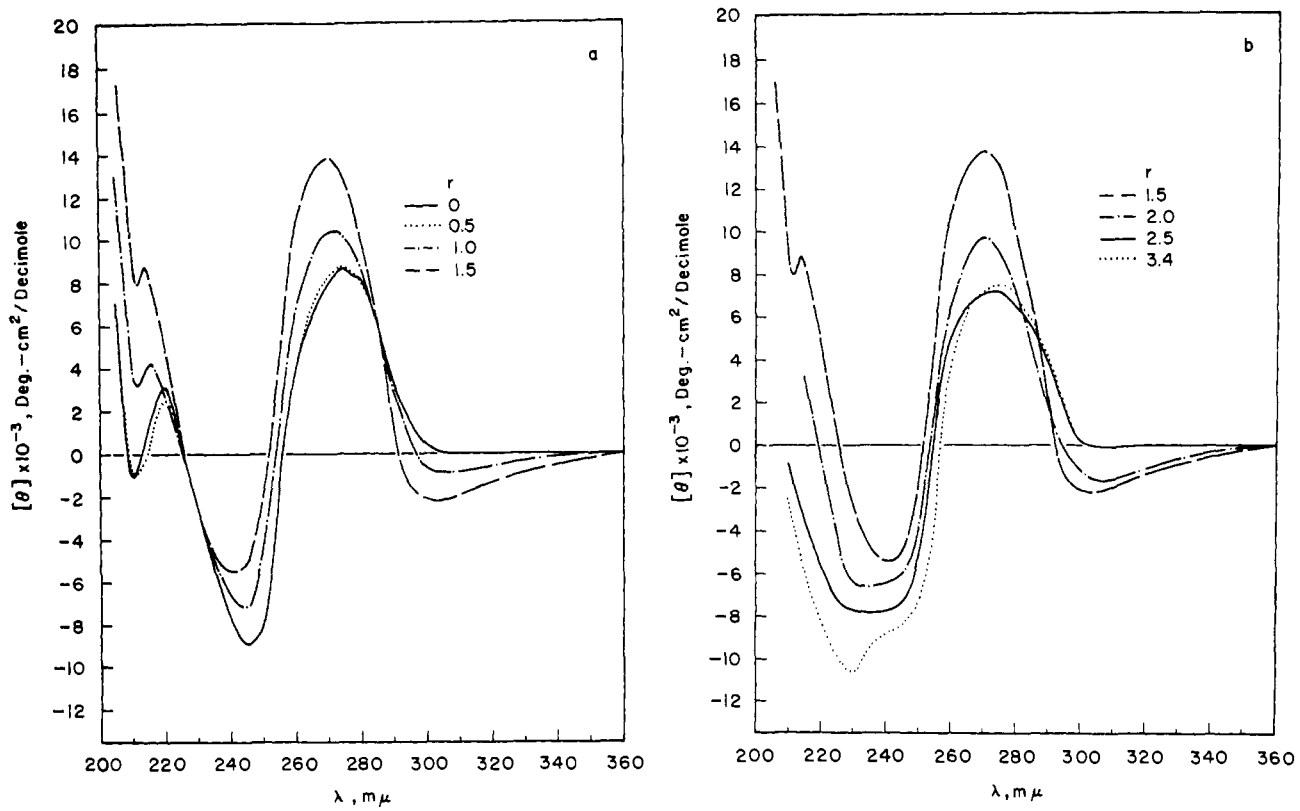

FIGURE 2: Circular dichroism spectra of histone IV–DNA complexes as a function of r, the histone:DNA ratio. Complexes were prepared by urea–salt-gradient dialysis by removal of urea at 0.15 M NaCl (dialysis A). Circular dichroism spectra were measured in 0.14 M NaF–0.001 M Tris (pH 7.0) at a DNA concentration of 1.5×10^{-3} or 1.5×10^{-4} M (moles of phosphate) with optical path length of 0.1 or 1 cm. Histone:DNA ratio (moles of residue/moles of phosphate) is as follows: in part a, —, 0; . . . . . , 0.5; —·—, 1.0; — —, 1.5; in part b, — —, 1.5; —·—, 2.0; —, 2.5; . . . . , 3.4.

bath), employing pure DNA as standard. Both methods agree very well. The molar extinction coefficient, ϵ_{260} (mole phosphate) = 6800, was used for the calculation of pure DNA concentrations. Stock solutions of histone were made up by weight, using samples dried to constant weight by drying in a high-vacuum desiccator for a period of 4 days. The mean residue weight of the HCl salt of histone IV was calculated to be 120.26 from the amino acid composition (DeLange *et al.*, 1969a). The mean residue extinction coefficient, ϵ_{278}, of histone IV in neutral pH was found to be 52.

Results

Changes of Circular Dichroism Spectrum of DNA as a Function of Histone:DNA Ratio for Complexes Prepared by Dialysis A. If DNA–histone complexes are prepared in a very specific manner (dialysis A) changes in the circular dichroism spectrum of DNA by complexing with the arginine-rich histone IV can be observed. The circular dichroism changes vary as a function of histone to DNA ratio as shown in Figure 2 (in 0.14 M NaF–0.001 M Tris, pH 7.0). Histone to DNA ratio, r, is expressed as the ratio of histone amino acid residues to DNA nucleotide residues. As r increases from 0 to 0.5, the circular dichroism spectrum is essentially unchanged ($[\theta]_{275} = 8490$, $[\theta]_{245} = -9200$). From ratios of 1.0–1.5 a gradual blue shift and an increasing amplitude of the 275-mμ positive circular dichroism band of DNA, to a value of $[\theta]_{270} = 13{,}200$, were observed. The 245-mμ negative ellipticity band is concurrently decreased in amplitude and apparently blue shifted ($[\theta]_{242} = -5700$). A new negative band centered at 305 mμ is generated. Sarkar *et al.* (1967) have reported a very weak negative band of DNA at 310 mμ. The changes induced by the complexes appear to be increasing the magnitude of this weak cicrular dichroism band. The magnitude of the positive ellipticity band at 220 mμ increases and blue shifts with increasing r ratios. The very large positive circular dichroism band of DNA at 189 mμ is also increased in magnitude at $r = 1.5$ as can be seen in Figure 3. If r becomes larger than 1.5, the magnitudes of the circular dichroism changes now decrease as seen in Figure 2b. The decrease in the spectral changes levels off at $r = 2.5$ and the circular dichroism spectrum is then close to that of DNA. However, increasing contributions from the histone spectrum can be seen below 245 mμ.

The circular dichroism spectra of the complexes in 0.14 M NaF–0.001 M Tris (pH 7.0) show no concentration dependence as can be seen in Figure 3 for $r = 1.5$ in a concentration range of 1.53×10^{-3} to 7.65×10^{-6} M, a 200-fold dilution. For the other ratios no concentration dependence was observed for the concentration range investigated, 1.5×10^{-4} to 1.5×10^{-3} M. This behavior is different from DNA complexes with a lysine-rich histone f1 which were extremely concentration dependent (Fasman *et al.*, 1970b). The histone IV complexes also appear to be very stable in 0.14 M NaF. The circular dichroism spectrum of the complex at $r = 1.5$ has been examined over a period of 2 weeks, and no change was noted. However, when the complexes are further dialyzed down to a lower ionic strength of 0.01 M NaF (in the absence of urea), the spectral changes slowly decrease in magnitude from $[\theta]_{270} = 13{,}200$ to 10,300, for the complex $r = 1.5$, over a

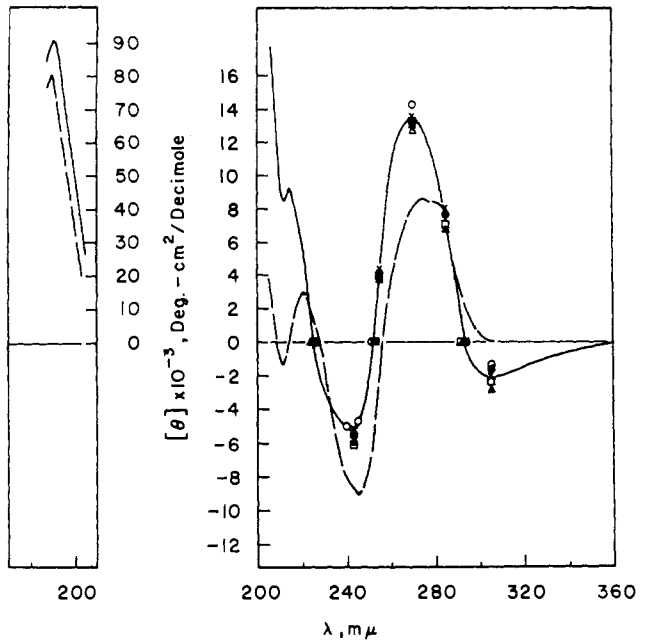

FIGURE 3: Circular dichroism spectra of histone IV–DNA at different concentrations. Complex at $r = 1.5$ was prepared by dialysis A. Circular dichroism spectra were measured in 0.14 M NaF–0.001 M Tris (pH 7.0) at the following DNA concentrations (moles of phosphate) and optical path lengths: ○, 1.53×10^{-3} M (0.1 cm); ●, 3.06×10^{-4} M (0.5 cm); ×, 1.53×10^{-4} M (1.0 cm); △, 3.06×10^{-5} M (5.0 cm); □, 1.53×10^{-5} M (5.0 cm); ▲, 7.65×10^{-6} M (5.0 cm). The very strong band at 189 mμ was measured at 1.50×10^{-3} M (0.1 mm). Solid line is spectrum of complex and dashed line is that of control DNA ($r = 0$).

period of 7 days. Thus there is ionic strength dependence on the stability of the complex but not upon the circular dichroism of the initially formed complex. Conversion of the conformation in 0.14 M NaF into that stable in 0.01 M NaF seems to be a very slow process. In 0.14 M NaF, the characteristic structure of the complexes is, therefore, very stable.

The averaged circular dichroism parameters of the histone–DNA complex at $r = 1.5$, which shows maximum changes, are listed in Table I, and are compared to the original DNA spectrum.

Effect of Sodium Dodecyl Sulfate on Histone–DNA Complexes. In a previous publication (Shih and Fasman, 1970), it has been found that the circular dichroism spectrum of chromatin is different from that of isolated DNA. By adding 0.1% SDS[1] to the chromatin solution, the DNA spectrum was restored to that of pure DNA, presumably by dissociation of proteins. The DNA spectrum is not affected by 0.1% SDS alone. A similar effect has also been observed for histone IV–DNA complexes as shown in Figure 4. $[\theta]_{275}$ and $[\theta]_{245}$ of the complex to which 0.1% SDS was added after dialysis A are similar to those of DNA. The altered circular dichroism spectra of the complexes thus disappear by dissociating the histone from the DNA.

Complexes without Change of the Circular Dichroism Spectrum of DNA. Characteristic changes of the circular dichroism spectrum of DNA by complexing with histone IV can only be observed when the complex is prepared in a very specific

TABLE I: Circular Dichroism Parameters of Histone IV–DNA Complex ($r = 1.5$) in Comparison to Those of DNA.[a]

	Band 1		Band 2		Band 3		Band 4		Band 5		Band 6	
	λ (mμ)	$[\theta]^b$	λ	$[\theta]^b$	λ	$[\theta]^b$	λ	$[\theta]^b$	λ	$[\theta]^b$	λ	$[\theta]^b$
DNA	310	0	275	8490 ± 280	245	−9200 ± 197	220	3090 ± 204	210.5	−1330 ± 302	189	75,200 ± 9800
Histone–DNA	304	−1950 ± 660	270	13,200 ± 660	242	−5700 ± 580					190	83,600 ± 14,100

[a] Averaged data from 10 preparations from dialysis A are presented with their standard deviation. Solvent is 0.14 M NaF–0.001 M Tris (pH 7.0). [b] (deg cm²)/dmole.

[1] Abbreviation used that is not listed in *Biochemistry* 5, 1445 (1966), is: SDS, sodium dodecyl sulfate.

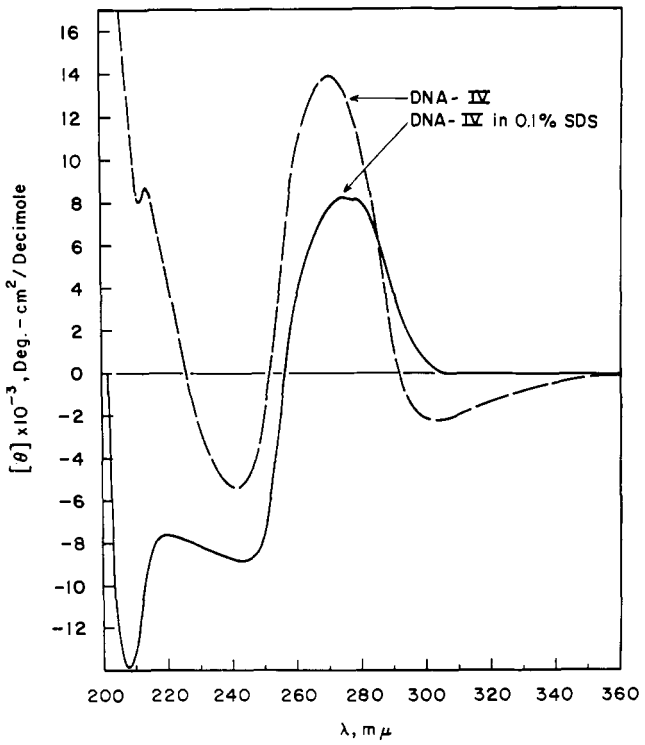

FIGURE 4: Effect of sodium dodecyl sulfate on the circular dichroism spectrum of the histone–DNA complex. Complex at $r = 1.5$ was prepared by dialysis A. Circular dichroism was measured in 0.14 M NaF–0.001 M Tris, pH 7.0 (----). —, spectrum of the same complex in 0.1% (w/v) SDS in the same solvent. Concentration is 1.12×10^{-4} M (moles of phosphate) with path length 1 cm.

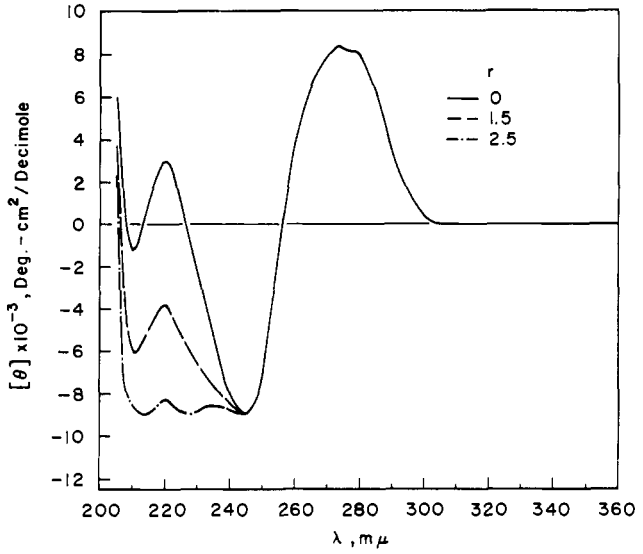

FIGURE 5: Circular dichroism spectra of histone IV–DNA complexes as a function of r, the histone:DNA ratio. Complexes were prepared by dialysis B. Complexes were prepared by urea-salt-gradient dialysis by removal of urea at 0.015 M NaCl. Circular dichroism spectra were measured in 0.01 M NaF–0.001 M Tris (pH 7.0). Concentrations were at 1.5×10^{-3} or 1.5×10^{-4} M with optical path length of 0.1 or 1 cm. Histone:DNA is as follows: —, 0; — —, 1.5; — · —, 2.5.

manner (dialysis A). If the salt-gradient dialysis in 5 M urea is carried out down to a lower ionic strength of 0.015 M NaCl, and urea is then removed at this point by dialysis against 0.015 M NaCl (dialysis B), no changes of the circular dichroism spectrum of DNA can be observed, as seen in Figure 5. Under these conditions, nevertheless, the histone is bound to DNA (Shih and Bonner, 1970). Even on varying the ratio to $r = 2.5$, no changes were observed. Conversion of this conformation to that found in Figure 2 cannot simply be achieved by adjusting the ionic strength back to 0.14 M NaF, either by directly adding concentrated NaF solution or dialysis against 0.14 M NaF. Several attempts have been made to cause this conformational change, that is, to dialyze the type of complex formed at 0.01 M NaF into 0.14 M NaF in the presence of 5 M urea, 20% (v/v) dioxane or 20% (v/v) dimethyl sulfoxide, and none resulted in a successful conversion into the other structures. The conformations of the complexes assumed at low ionic strength formed in the presence of urea are therefore very stable and not interconvertible by the procedures described. The significance of this point will be further discussed later.

It appears, then, that the concentration of the salt at which urea is removed during the process of salt-gradient dialysis is very important for forming a specific type of complex. A series of experiments has been carried out by removing the urea at different salt concentrations during a salt-gradient dialysis. The result shows that changes of the circular dichroism spectra for a histone:DNA ratio of 1.5 are most pronounced by removal of urea at 0.15 M NaCl. Smaller changes in the DNA circular dichroism are observed by removing urea at 0.3 M but no change was found at 0.6 M or higher.

Complexes prepared by a salt-gradient dialysis without urea from 2 to 0.15 M NaCl also show no change in the circular dichroism spectrum of the DNA. A great deal of the histone is also lost by this procedure (absence of expected histone circular dichroism contribution); presumably histone IV precipitates out of solution due to aggregation at high salt concentration.

Simple mixing experiments under vigorous stirring have been carried out by adding histone IV dropwise to a DNA solution until histone:DNA ratios of 1.5 and 2.5 were reached. At both ionic strengths of 0.01 and 0.14 M NaF, no change in the circular dichroism spectrum of DNA has been observed. This agrees with the work of Wagner (1970) on f2al–DNA complexes.

It is well known that nucleohistone is least soluble at the isotonic ionic strength of 0.15 M (Zubay and Doty, 1959). The specific conformational change of DNA produced by complexing with histones may be the result of the specific association state of the DNA–histone complexes produced by removal of urea at 0.15 M NaCl. This specific association state is not only dependent on the salt concentration, but also on the histone:DNA ratio of the complex. At r higher than 1.5 $\sim$ 2.0, the specific association of the complexes appears to be interfered with, and therefore the complexes do not show circular dichroism changes. The dependence of the final circular dichroism spectrum upon the concentration of urea during dialysis was also investigated. Removal of the initial 5 M urea in steps in the presence of 0.15 M NaCl, *i.e.*, 5, to 3, to 1, to 0 M yielded complexes showing no circular dichroism change. Hence the gradient of urea removal is also important. A tentative explanation is the following. The association of the histone IV with the DNA, in 0.15 M NaCl in the presence of 5 M urea, is initiated (charge attractions) but total binding is still incomplete and there is no intercomplex association. On lowering the urea concentration complete binding of histone IV and DNA occurs (*e.g.*, hydrophobic and hydrogen bonds)

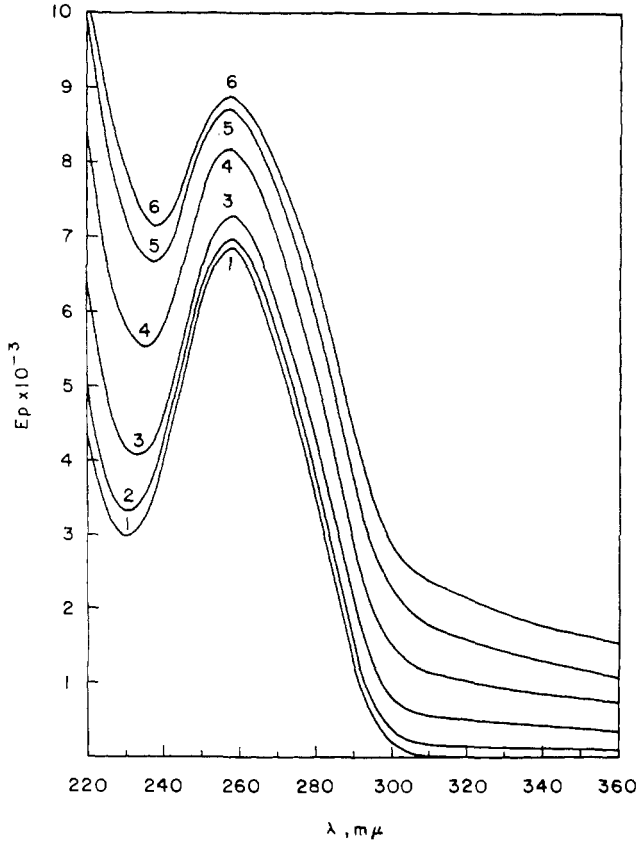

FIGURE 6: Ultraviolet absorption spectra of histone IV–DNA complexes. The samples as in Figure 2 (prepared by dialysis A) were measured at 1.5 × 10^{-4} M (moles of phosphate) in 1-cm path-length cells. Histone:DNA ratio is as follows: 1, 0; 2, 0.5; 3, 1.0; 4, 1.5; 5, 2.0; 6, 2.5.

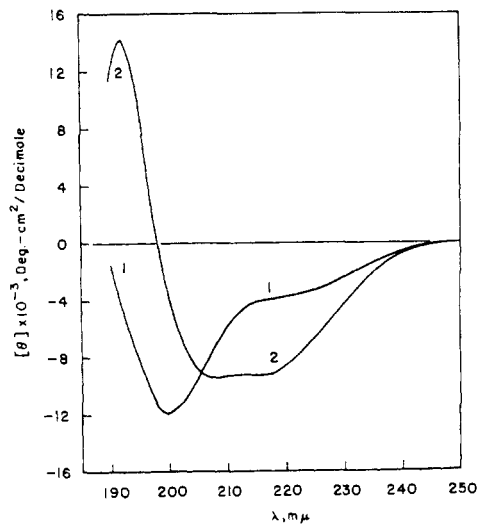

FIGURE 7: Circular dichroism spectra of histone IV. Spectra of free histone measured at different ionic strength (1), 0.01 M Tris (pH 7.0), or (2), 0.14 M NaF–0.002 M Tris (pH 7.0). Histone amino acid residue concentration was 4.10 × 10^{-3} or 8.20 × 10^{-3} M and optical path lengths are 0.2, 0.5, and 1.0 mm for the former and 0.1, 0.5, and 1.0 mm for the latter.

and simultaneous association of these complexes. However, upon removal of urea in steps, the relative rates of total binding and association are different than in the one step gradient. Therefore, the same specific state of association is not achieved in the two methods. A factor which may play a role for this conformational change, as pointed out previously (Fasman *et al.*, 1970b), is dehydration of the complex at high ionic strength. This water removal may be necessary for histone molecules to be tightly bound to the DNA surface in order to form a specific aggregate.

Possible Circular Dichroism Changes Due to Artifacts. It is appropriate to examine the possibility that the observed circular dichroism spectral changes might be due to a simple light-scattering artifact as pointed out by some workers (Urry *et al.*, 1970). Several experimental observations argue against this possibility: (1) The circular dichroism spectra of the complex measured at different concentrations in a constant path length (different turbidity) appear to be identical within experimental error (Figure 3), (2) although samples showing altered circular dichroism spectra are slightly turbid, not all turbid samples show altered spectra. For example, complexes made from salt-gradient dialysis without urea are much more turbid than with urea, yet no changes in the circular dichroism spectrum of DNA are observed in the former, and are seen in the latter. Further, from ultraviolet absorption spectra as shown in Figure 6, the light scattering increases as r becomes greater from 0 to 2.5, while the circular dichroism spectra actually show maximum change at r = 1.5. (3) The first negative ellipticity band at 305 mμ is skewed toward the long-wavelength side (Figure 2a, r = 1.5), and thus appears like a scattering phenomenon. However, at higher values (Figure 2b, r = 2.5 and 3.4) where turbidity is increased, this band disappears. The very weak circular dichroism band at 310 mμ for DNA and the much stronger band at 295 mμ for RNA, which are measured for clear solutions, also appear similar to the band in the complex at r = 1.5 (Sarkar *et al.*, 1967; Samejima, *et al.*, 1968). (4) Many laboratories including ours have done control experiments by using artificial scattering media, *e.g.*, colloid sulfur or alumina, to cause a turbidity much higher than the complexes display, yet no distortion of circular dichroism or optical rotatory dispersion spectra of solvent or DNA was observed (Gratzer and McPhie, 1966; Cohen and Kidson, 1968; B. Davidson, personal communication, 1970). Thus the circular dichroism spectral alteration seems to be due to the intrinsic optical properties of a specific DNA–histone complex.

Conformation of Free Histone IV. The conformation of histones has been the subject of many spectroscopic studies (Bradbury *et al.*, 1962, 1965; Jirgensons and Hnilica, 1965; Tuan and Bonner, 1969; Jirgensons, 1969; Tamburro *et al.*, 1970; Boublik *et al.*, 1970; Wagner, 1970). These studies are all concerned with purified fractions, usually contaminated to some degree. The circular dichroism spectra of a homogeneous molecular species of histone IV are reported herein (Figure 7). The circular dichroism of histone IV in low ionic strength solutions, *i.e.*, distilled water, 0.01 M Tris (pH 7.0) or 0.01 M NaF, are similar to one another. The conformation of the histone in these media is essentially a random coil, although the negative band at 200 mμ is of smaller magnitude and slightly red shifted as compared to synthetic poly-L-amino acids (reviewed by Timasheff and Gorbunoff, 1967) and possesses a shoulder at 215 to 230 mμ. It is of interest that these spectral features are very similar to the circular dichroism spectra of polypeptide films in the random conformation, in which the random coil is subjected to steric constraints, and similar to many denatured proteins (Stevens *et al.*, 1968; Fasman, *et al.*, 1970a). In 0.14 M NaF, the conformation of histone IV is quite different

from that found in low ionic strength. The circular dichroism spectrum is that of a protein containing considerable α-helical conformation. If one assumes that there is no β structure, the helical content would be about 40%. The spectrum, however, can also fit to the synthetic spectrum with 24% α-helix, 40% random coil, and 36% β structure (Greenfield and Fasman, 1969). In 0.14 M NaF, histone IV is aggregated as judged by the presence of light scattering observed in the ultraviolet absorption spectrum. The circular dichroism spectrum also changes slightly on standing in this ionic strength. The time effect seems to cause an increase in β structure, as the negative ellipticity band of the β structure at 217 mμ becomes more pronounced. Such changes of histone conformation as a function of ionic strength, except for the lysine-rich histone f1, are in agreement with many earlier observations by other workers as cited above.

An interesting high-resolution nuclear magnetic resonance spectroscopy study indicates that the C-terminal half of the histone IV molecules (apolar half) is involved in aggregation, and may be the region for the α-helix formation (Boublik *et al.*, 1970).

Conformation of Histone IV Bound to DNA in Its Native Conformation Produced by Dialysis B. DNA–histone complexes prepared by dialysis B show no change in DNA conformation, as $[\theta]_{275}$ and $[\theta]_{245}$ are unchanged (Figure 5). This offers the opportunity to study the histone conformation when it is bound to the DNA in its native conformation. The spectral difference between the circular dichroism spectra of the complex and control DNA is the contribution from the histone component and this is seen in Figure 8. It is very interesting to note that the histone conformation loses its salt sensitivity when histone is attached to DNA, as shown in Figure 8. As stated earlier, the circular dichroism spectra of these complexes are not changed by adjusting the ionic strength from 0.01 to 0.14 M NaF. This is in strong contrast to what is observed for the free histone (Figure 7). Strong interaction of the histone and the DNA thus appears to be able to determine the conformation of the histone molecule bound to DNA. The histone conformation, constrained by the DNA, is therefore unable to respond to changes of ionic strength. The conformation that the histone assumes on the DNA, as judged from the circular dichroism spectra, is qualitatively similar to histone in 0.14 M NaF, namely, it contains considerable α-helical structure. However, differences are apparent. This is similar to the work of Wagner (1970) on f2a1 binding to DNA, although there the binding was produced by mixing the components directly. It is possible that it assumes a distorted α helix, although β structure cannot be ruled out by the circular dichroism spectra. However, this is the conformation of histone IV under conditions where the DNA conformation remains unchanged. When the DNA conformation changes, no estimate of histone conformation is possible.

In connection with this point, chromosomal proteins of chromatin also show considerable α-helical content as studied by circular dichroism or optical rotatory dispersion (Oriel, 1966; Permogorov *et al.*, 1970; Simpson and Sober, 1970; Shih and Fasman, 1970). However, due to the presence of non-histone proteins (*ca.* 40% of the proteins) and changes of DNA conformation in the native complex, accurate estimation of α-helical content of histones *in situ* is not certain.

Discussion

Conformational Change of DNA in Complexes with Histone IV. A remarkable feature of the circular dichroism spectrum of DNA is its conservative nature, *i.e.*, nearly similar magnitudes of the 275-mμ positive band and 245-mμ negative band with crossover point near the 260-mμ absorption maximum (reviewed by Yang and Samejima, 1969). Tinoco, from exciton theory, interpreted this type of spectrum to be the result of base stacking nearly perpendicular to the helical axis of the B form of DNA (Tinoco, 1964, 1968). In double-stranded or hairpin single-stranded RNA, the molecular geometry differs from that of DNA by a larger base tilting of 15°. According to this theory, this tilting would result in the nonconservative spectrum observed in transfer RNA, rRNA, and tobacco mosaic virus RNA (Brahms and Mommaerts, 1964; Sarkar *et al.*, 1967), and double-stranded rice dwarf virus RNA (Samejima *et al.*, 1968). Under a stringent annealing condition (dialysis A), a specific DNA complex with histone IV is formed, which has an altered circular dichroism spectrum indicating a conformational change of the DNA. Maximum changes in the circular dichroism spectrum of DNA occur at a histone:DNA ratio of 1.5 and the circular dichrosim spectrum is remarkably similar to that of RNA. This circular dichroism spectrum is marked by the enhancement of the very weak negative band at 310 mμ of DNA, increased amplitude and blue shift of the 275-mμ positive band, and decreased amplitude of the 245-mμ negative band. The spectrum is therefore nonconservative. Although the strong negative band of RNA at 210 mμ is not evident in the DNA–histone complexes, it is quite possible this may be a particular feature of RNA or may be buried in the very strong, enhanced, and red-shifted 189-mμ positive band of the complex. The theoretically calculated circular dichroism spectrum of the A form of DNA, which has the bases inclined 20° to the axis (Johnson and Tinoco, 1969), and the circular dichroism spectrum of a DNA film

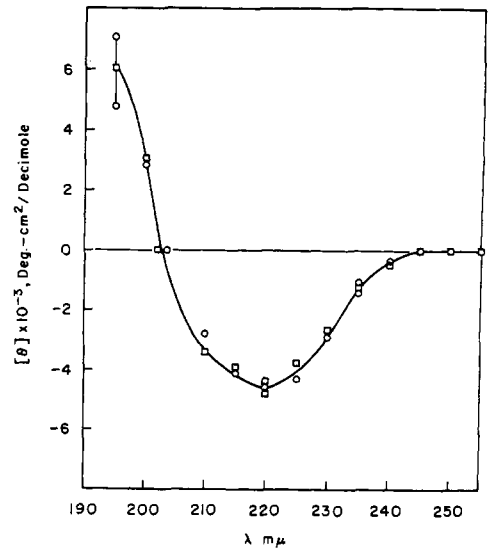

FIGURE 8: Circular dichroism spectra of histone IV bound to the native conformation of DNA. Histone IV–DNA complexes were prepared by dialysis B and show no change in DNA spectra (Figure 5). Circular dichroism spectra of complexes at $r = 1.5$ or 2.5 were subtracted from the circular dichroism of control DNA ($r = 0$) measured at a similar concentration of 1.5×10^{-4} M, with optical path length of 1.0 or 0.5 cm. $[\theta]$ based on amino acid residue concentration. Solvents are: □, 0.01 M NaF–0.001 M Tris (pH 7.0) and ○, 0.14 M NaF–0.001 M Tris (pH 7.0). These complexes were dialyzed back to 0.14 M NaF from 0.01 M NaF. Error bars represent range of experimental error.

in the A form (Tunis-Schneider and Maestre, 1970) are in very good agreement with the present circular dichroism observations.

The change in the magnitude of $[\theta]_{270}$ of the complex at r = 1.5, compared to DNA, is only about 30% of the difference between RNA and DNA in this wavelength region. The shift to the blue of this ellipticity band is also about 30% of that shift between RNA at 261 mμ and DNA at 275 mμ. However, as only 25% of the DNA is complexed with histone molecules at r = 1.5, as calculated from the biphasic melting profiles of the complexes (Shih and Bonner, 1970), one would not expect complete conversion into the A form. The ratio of cationic amino acid residues of lysine, arginine, and histidine to that of nucleotide residue at r = 1.5 is 0.38. Thus perhaps the DNA in this complex is 30% in the A form, while the remainder retains the B form.

The possibility that the observed circular dichroism changes might be due to some form of long-range ordered structure of the associated complex rather than altered base stacking of the DNA helix cannot be completely ruled out by a circular dichroism study alone. However, in light of all the evidence presented, as well as that reported for chromatin (Shih and Fasman, 1970), it is very likely that the circular dichroism spectral changes reflect changes in DNA conformation.

Conformation of Histone IV Bound to DNA in Its Unaltered Conformation. In understanding the structural basis of DNA–histone interactions, an interesting problem is what conformation the histone assumes upon binding to DNA. In this simple system involving DNA complexes with a homogeneous histone species, direct observation is possible for those complexes where the DNA structure remains unaltered. The complexes produced by dialysis B in low ionic strength are soluble and conformation of the DNA is unchanged; thus information about the conformation of the histone bound to DNA can be obtained. Interaction with DNA appears to exert a strong influence on what conformation the protein molecule assumes. The conformation of the free histone in the dialysis mixture (*i.e.*, in the presence of urea) is probably different than in aqueous solution. However, the final conformation of the histone is determined by its binding to DNA. It is interesting that the histone conformation is fixed by DNA and does not respond to changes in ionic strength of the solution once the complex is formed. The circular dichroism spectrum of histone IV bound to DNA indicates that it has assumed some α-helical structure as visible in Figure 8, although the expected double ellipticity bands for the α helix (Greenfield and Fasman, 1969) are not clearly visible. A possibility still exists that some helical segments may be distorted by its binding to DNA in order to assume the curvature of the DNA groove surface. Similar studies by Wagner (1970) indicated a helical content of 23% although the method of complex formation differed. However, if the histone alters the conformation of the DNA (as with specific annealing condition dialysis A) then the histone conformation is probably different from that observed here (with dialysis B). From the amino acid sequence data, the distribution of cationic side chains is not random (DeLange *et al.*, 1969a). The distribution of the cationic amino acid residues is such that if some α-helical conformation is assumed in the N-terminal-half of the molecule, the more polar end of the polypeptide, these residues are in good positions to interact with DNA phosphate groups (Shih and Bonner, 1970). In the free histone, the apolar half of the molecule, C terminal, seems to have a greater potential for α-helix formation (Boublik *et al.*, 1970), In view of the strong interaction between DNA and histone, the location of these helical segments remains an interesting problem.

The circular dichroism changes induced in the DNA spectrum by complexing with histone IV are different from those induced by f1(I) (Fasman *et al.*, 1970b). The latter shows a progressive decreasing and red-shifted 275-mμ ellipticity band and increasing 245-mμ band upon increasing the histone:DNA ratio, while histone IV causes an increase and blue shift in the 275-mμ band, a decrease in the 245-mμ band, and an increase in the negative 305-mμ band, at r = 1.5, and these revert to their original values at higher r values. Therefore, the conformational alteration of DNA seems to be different with these two histones. From the primary structures of these two histones (DeLange *et al.*, 1969a; Bustin and Cole, 1970), there is a pattern of clustering of cationic residues; however, they occur in different regions of the molecule, *e.g.*, histone IV (f2a1) at the N-terminal half and histone f1(I) at the C-terminal half, including most of the proline for the latter. These portions of the histone molecules have been implied to be the major DNA binding sites. It is quite possible that the structure of f1 bound to DNA is different from that of histone IV. This together with the different sequences and distribution of apolar residue (which may interact specifically through hydrophobic interactions with the bases) may account for the different conformational changes of DNA induced by these two histones.

Implication for Structure and Function of Eukaryotic Chromosomes. The conformation of DNA in chromatin has been shown to be different from that of isolated DNA in solution due to its association with chromosomal proteins (Shih and Fasman, 1970, and references cited herein). By studying synthetic complexes of DNA with the lysine-rich histone f1, the DNA conformation was shown to be changed (Fasman *et al.*, 1970b). In this paper it is further demonstrated that the conformation of DNA can also be altered by complexing with the arginine-rich histone IV under specific conditions of complex formation. Histone f1(I) accounts for 20.6% ($r \simeq$ 0.6) and histone f2a1 (IV) for 16.3% ($r \simeq$ 0.5) of the total histone from calf thymus (Panyim and Chalkley, 1969). How the observed different circular dichroism changes induced by these purified histones contribute to the circular dichroism of chromatin is not quite clear. However, it is possible to obtain further insight into the structure and function of chromatin by studying these simple reconstituted systems with increasing complexity, by combination of different histones and with some other chromosomal non-histone proteins and RNA.

The extreme conservation of the primary structure of histone IV in the two evolutionarily distant organisms of cow and pea does indicate the essential functions histones play in the cell nucleus (DeLange *et al.*, 1969b). The stringent structural requirement of this histone molecule renders most mutations to be lethal and not observable. This would suggest that histone molecules may be involved in multiple functions, *e.g.*, binding to DNA, interaction with other histone molecules, other chromosomal proteins and RNA, etc. In the biological sense, histones may play both a regulatory role of genome expression and a role in maintaining structural integrity of chromosomes.

An interesting observation of this study is the plasticity of DNA–histone complexes. Complexes prepared under a very specific annealing condition yield a characteristic structure which differs from the equilibrium structure observed at a lower ionic strength. These structures are very stable

and not easily interconvertible. Other conditions of binding produce no alteration in DNA structure. Eukaryotic chromosomes have long been observed to undergo a very characteristic morphological change during cell division as documented in the classical treaties of Rhoades (1961) and Mazia (1961). The present observation of the plasticity of DNA–histone complexes may provide the molecular approach and basis for understanding this complex biological phenomenon.

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