Circular Dichroism Studies of Calf Thymus Ca^{2+} Nucleohistone IV†

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ABSTRACT: The conformational properties of a nucleohistone complex composed of calf thymus histone IV and DNA have been studied by circular dichroism spectroscopy. The Ca^{2+} nucleohistone IV complex was prepared by annealing during $CaCl_2$ gradient dialysis in the presence of 5 M urea. The conformational characteristics of this nucleohistone are shown to be dependent upon the $CaCl_2$ concentration at which the nucleohistone is removed from urea. Removal of Ca^{2+} nucleohistone IV from urea at 0.15 M $CaCl_2$ yields a complex in which the DNA component retains the conformation of purified DNA while removal of the nucleohistone from urea at 0.05 and 0.005 M $CaCl_2$ yields a complex which shows marked changes in both its DNA and histone conformation. The nonconservative circular dichroism spectrum of Ca^{2+} nucleohistone IV prepared at 0.05 M $CaCl_2$ is characteristic of the polynucleotide conformation assumed by double-stranded RNA and DNA in the A form. The circular dichroism spectrum of Ca^{2+} nucleohistone IV prepared at 0.005 M $CaCl_2$ is quite similar to the circular dichroism spectrum of the DNA component of isolated interphase chromosomes (chromatin). This observation suggests Ca^{2+} nucleohistone IV as a structural model for chromatin.

The chromosome of higher order (eucaryote) species functions not only as a source of genetic information but also to control the availability of this information for biological expression. The transcriptional control function of the eucaryote chromosome is of particular interest because it differentiates the eucaryote genetic system from the more well understood viral and bacterial genetic systems. The eucaryote chromosome is composed of DNA in close association with histones, nonhistone protein, and chromosomal RNA. Essential to an understanding of the genetic functioning of this complex chromosomal material is a firm knowledge of its structure. Although much generalized structural information regarding the eucaryote chromosome has been gathered from direct studies of isolated interphase chromosomes (Oriel, 1966; Tuan and Bonner, 1969; Sponar *et al.*, 1970; Permogorov *et al.*, 1970; Simpson and Sober, 1970; Shih and Fasman, 1970; Henson and Walker, 1970; Wilhelm *et al.*, 1970; Wagner and Spelsberg, 1971; Ohlenbusch *et al.*, 1967; Ohba, 1966; Bradbury and Crane-Robinson, 1964; Wilkins *et al.*, 1959; Pardon *et al.*, 1970; Bram, 1971), a more detailed structural analysis is facilitated by the study of a markedly simplified model for chromosomal material. In the present study a well-defined complex between DNA and histone IV (nucleohistone IV) (Shih and Fasman, 1971; Li *et al.*, 1971) has been chosen as a chromosomal model. Nucleohistone IV serves as a particularly good chromosomal model for the study of chromosomal DNA conformation. Recent studies suggest that histone IV may have a major role in the determination of chromosomal DNA conformation (Wagner and Spelsberg, 1971). The conformation of the DNA component of isolated chromosomal material appears to be related to the transcriptional control function of eucaryote chromosomes (Wagner and Spelsberg, 1971) suggesting a conformational mechanism (Shih and Fasman, 1970) for this centrally important function of the genetic system of higher order species. This observation suggests that a detailed knowledge of the structure of nucleohistone IV may shed light upon the structure–functional relationships within the eucaryote chromosome. Also, analysis of experimental observations of nucleohistone IV is facilitated by knowledge of the amino acid sequence of histone IV (DeLange *et al.*, 1969; Ogawa *et al.*, 1969).

The natural milieu of the nucleus includes a significant concentration of divalent metal ions (Mirsky and Osawa, 1961). A decrease in the calcium ion concentration of isolated nuclei has been demonstrated to cause swelling of nuclear chromatin, nuclear rupture, and a dramatic change in the circular dichroism spectrum of the DNA component of nuclear chromatin (Wagner and Spelsberg, 1971). These results warrant a further investigation into the structural role of calcium ion in native chromosomal material. In the present investigation the effects of calcium ion on the structure of the chromosomal model, nucleohistone IV, have been studied. Marked structural effects due to divalent calcium ion have been observed.

Material and Methods

Arginine-rich histone IV was prepared from calf thymus tissue. Whole histone was isolated from crude chromatin (Shih and Bonner, 1969) by extraction with 0.4 M HCl. It was then further purified on carboxymethylcellulose. Whole crude histone in 0.225 M LiCl (pH 5.5) was applied to a column of carboxmethylcellulose equilibrated with 0.05 M LiOH–acetic acid–0.225 M LiCl (pH 5.5), washed with 0.05 M ammonium bicarbonate, and eluted with 0.2 M HCl. Purified whole histone prepared in this manner was fractionated into the five histone components by gel filtration chromatography on Bio-Gel P-10 according to the procedure of Sung and Dixon (1970). The slowest moving component from the Bio-Gel P-10 chromatographic separation containing histone IV was recovered and rechromatographed on Bio-Gel P-60. The elution diagrams from the Bio-Gel P-10 and P-60 chromatographic runs are presented in Figure 1. Histone IV purified in this manner moved as a single band on polyacrylamide gel electrophoresis (Bonner *et al.*, 1968) and displayed the same electrophoretic mobility as the histone IV component of whole histone. Amino acid analysis revealed the same amino acid

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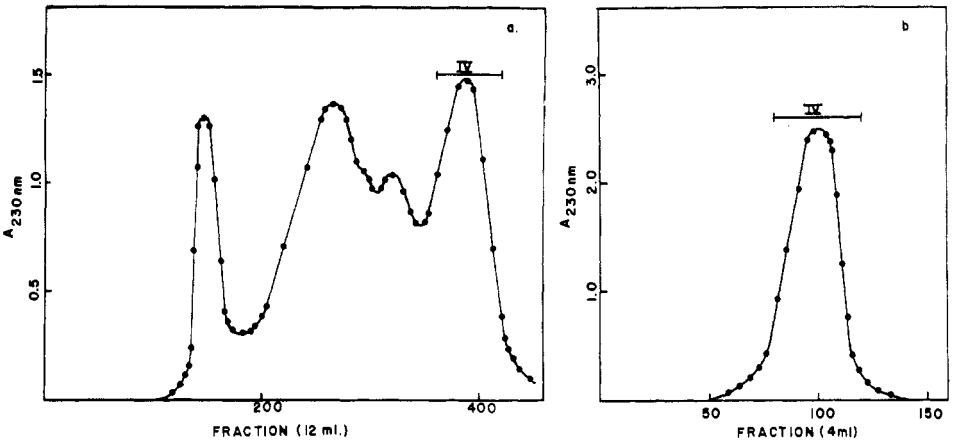

FIGURE 1: Gel filtration chromatography of calf thymus histones and the purification of histone IV. (a) Whole calf thymus histone was dissolved in 8 M urea–0.01 M HCl for 2 days at 4°. After a single 2-hr dialysis against 0.01 M HCl, about 1 g of whole histone was applied to a Bio-Gel P-10 column of the size 5 × 350 cm. The column was then eluted with 0.01 M HCl saturated with $CHCl_3$ at room temperature. (b) The fraction containing histone IV was lyophilized (approximately 200 mg) and rechromatographed on a Bio-Gel P-60 column of the size 2.8 × 120 cm. The center of the single fraction which contained histone IV was lyophilized to its HCl salt and kept at −20°.

composition for this histone as has been previously observed for histone IV (DeLange *et al.*, 1969; Ogawa *et al.*, 1969).

Calf thymus DNA, purchased from Worthington Biochemical Corp., was further purified by standard ethanol and 2-propanol precipitation procedures. The DNA concentration in the free state was determined from the absorbance at 260 nm, using ϵ_{260} 6500 M^{-1} cm^{-1}/nucleotide. The DNA concentration in nucleohistone IV complexes was determined from the absorbance at 260 nm in a solution 2 M in NaCl, using ϵ_{260} 6500 M^{-1} cm^{-1}/nucleotide; (*i.e.*, solutions of absorbance 3.0 used in circular dichroism (CD) measurements were diluted 10-fold with a NaCl solution of appropriate concentration to yield a final 2 M NaCl solution. In 2 M NaCl the complexes dissociate and light scattering due to turbidity is removed.) These determinations were checked by measuring the absorbance of the nucleohistone at 260 nm in 0.1% sodium dodecyl sulfate (Shih and Fasman, 1971).

Nucleohistone IV containing a histone IV amide residue to DNA nucleotide residue ratio of 2:1 was prepared by a $CaCl_2$ gradient dialysis in the presence of 5 M urea at 4°. A step gradient of 1.0, 0.9, 0.6, 0.3, 0.15, 0.05, and 0.005 M $CaCl_2$ in 5 M urea was used. Each dialysis step was carried out for 3 hr. Nucleohistone IV was removed from the step gradient at 0.15, 0.05, and 0.005 M $CaCl_2$. These nucleohistone IV complexes in 5 M urea–$CaCl_2$ were then exhaustively dialyzed against 0.15, 0.05, or 0.005 M $CaCl_2$ in 0.001 M Tris-HCl (pH 7.0) in order to rapidly remove the urea. Controls containing only histone IV or DNA were carried through identical dialysis procedures. No charge in the circular dichroism spectrum of DNA or histone IV due to these dialysis procedures was observed. Gel filtration chromatography was used in order to demonstrate binding between histone IV and DNA in the Ca^{2+} nucleohistone IV complexes described herein. The elution volumes of DNA, histone IV, and Ca^{2+} nucleohistone IV prepared at 0.15 M $CaCl_2$ were measured on an analytical Bio-Gel P-60 column equilibrated and eluted with 0.15 M $CaCl_2$–0.001 M Tris (pH 7.0). These studies were carried out on solutions identical with those used for CD studies. DNA and Ca^{2+} nucleohistone IV prepared at 0.15 M $CaCl_2$ both were eluted from the column as a single symmetrical peak at 9.8 ml while histone IV was eluted at 40.6 ml. These results show conclusively that a true complex exists at 0.15 M $CaCl_2$ between histone IV and DNA when prepared as described previously. It is assumed that complexes prepared at lower $CaCl_2$ concentrations are also completely bound.

Circular dichroism spectra were measured using a Durrum-Jasco J-20 circular dichroism spectrometer. Measurements were performed at 25° under a nitrogen atmosphere in fused quartz cells with an optical path length of 0.1 cm. The absorbance at 260 nm of the DNA samples and nucleohistone complexes was about 0.3 in all cases. The spectrometer was operated with an automatic slit program which maintains a constant spectral bandwidth of 16 Å. Spectra were measured at a time constant of 1 sec and a scanning speed of 5 nm/min. Mean residue ellipticity, $[\theta]$, is reported in (deg cm^2)/dmole on the basis of DNA nucleotide concentration.

Ultraviolet absorptions spectra were measured using a Cary 14 recording spectrophotometer operating at 25°.

Results

Effects of Ca^{2+} Ion Concentration on the Circular Dichroism Spectra of Free DNA and Histone IV. Shown in Figures 2–4 are the circular dichroism spectra of free calf thymus DNA and histone IV in 0.005, 0.05, and 0.15 M $CaCl_2$ at pH 7.0 buffered in 0.001 M Tris. These figures also include the circular dichroism spectrum of calf thymus DNA in the absence of Ca^{2+}. The circular dichroism spectrum of DNA in 0.001 M Tris in the absence of Ca^{2+} (Figures 2–4) is consistent with the spectra reported by a number of others (Brahms and Mommaerts, 1964; Sarkar *et al.*, 1967; Fasman *et al.*, 1970) for DNA in mild aqueous media.

The presence of Ca^{2+} has been shown to alter the positive DNA circular dichroism band at 275 nm (Cheng, 1965; Simpson and Sober, 1970). In the present study DNA carried through the $CaCl_2$ gradient dialysis in 5 M urea to $CaCl_2$ concentrations of 0.15, 0.05, and 0.005 M $CaCl_2$ displayed markedly similar spectra (Figures 2–4) with a positive band at 275 nm which was about 40% less intense than the same circular dichroism band in the spectrum of DNA at 0.0 M $CaCl_2$. Below 250 nm the circular dichroism spectra of DNA in $CaCl_2$ were not significantly different from DNA in mild aqueous media.

The circular dichroism spectra of free histone IV in 0.15,

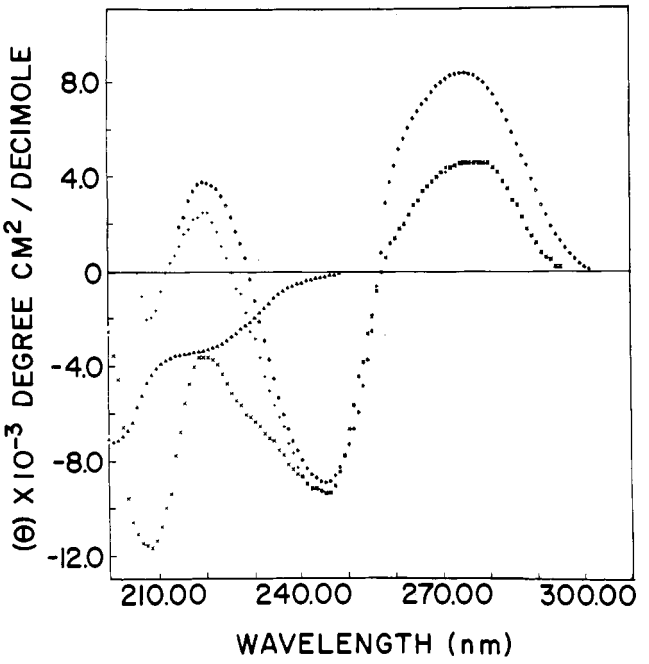

FIGURE 2: Circular dichroism spectra of DNA, histone IV, and nucleohistone IV in 0.15 M $CaCl_2$: (△) histone IV in 0.15 M $CaCl_2$–0.001 M Tris (pH 7.0); (□) DNA in 0.001 M Tris (pH 7.0); (+) DNA in 0.15 M $CaCl_2$–0.001 M Tris (pH 7.0); (×) nucleohistone IV prepared by $CaCl_2$ gradient dialysis in 5 M urea terminated at 0.15 M $CaCl_2$ in 0.15 M $CaCl_2$–0.001 M Tris (pH 7.0).

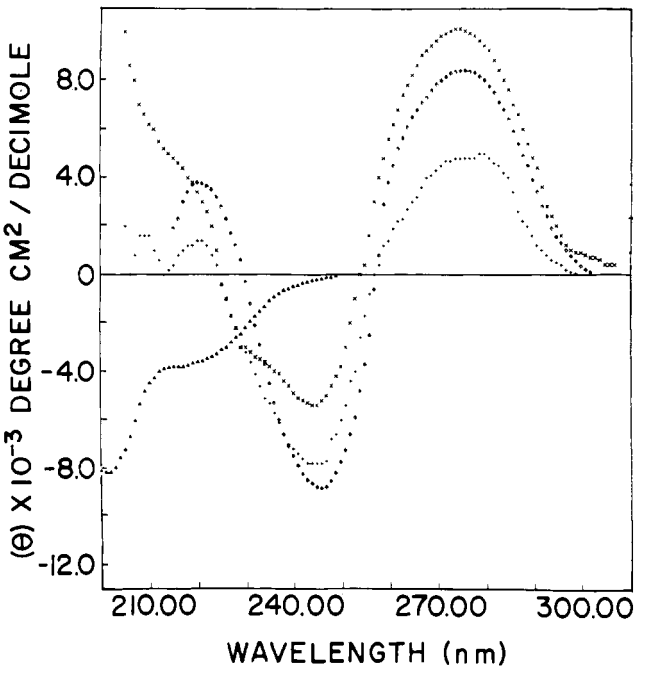

FIGURE 3: Circular dichroism spectra of DNA, histone IV, and nucleohistone IV in 0.05 M $CaCl_2$: (△) histone IV in 0.05 M $CaCl_2$–0.001 M Tris (pH 7.0); (□) DNA in 0.001 M Tris (pH 7.0); (+) DNA in 0.05 M $CaCl_2$–0.001 M Tris (pH 7.0); (×) nucleohistone IV prepared by $CaCl_2$ gradient dialysis in 5 M urea terminated at 0.05 M $CaCl_2$ in 0.05 M $CaCl_2$–0.001 M Tris (pH 7.0).

0.05, or 0.005 M $CaCl_2$ buffered in 0.001 M Tris (pH 7.0) (Figures 2–4) are essentially the same displaying two negative circular dichroism bands at 223 nm ($[\theta]_{223}$ −3500) and at 202 nm ($[\theta]_{202}$ −12,000) suggestive of a largely random coil protein containing a small α-helical contribution.

Effects of Ca^{2+} Ion Concentration on the Circular Dichroism Spectrum of Nucleohistone IV. The DNA region (above 240 nm) of the circular dichroism spectrum of nucleohistone IV removed from the $CaCl_2$ gradient dialysis at 0.15 M $CaCl_2$ (Figure 2) appeared indistinguishable from DNA in 0.15 M $CaCl_2$. In the region of the spectrum of nucleohistone IV in 0.15 M $CaCl_2$ (Figure 2) below 240 nm a small negative shoulder at 225 nm and a negative band at 208 nm ($[\theta]_{208}$ −11,800) are observed. These bands have been assigned to the peptide carbonyl transition of the amide residues of histone IV. The very small contribution to the ellipticity at 225 nm suggests that any α-helical contribution to the protein spectrum is relatively insignificant. The circular dichroism spectrum of nucleohistone IV at 0.15 M $CaCl_2$ describes a nucleohistone complex in which the DNA conformation appears to be independent of bound histone. Under the conditions of complex formation and in the medium of 0.15 M $CaCl_2$ the histone IV component of nucleohistone IV neither alters the conformation of the DNA component of the nucleohistone nor shields the DNA from salt effects due to calcium ion.

While continuation of the $CaCl_2$ gradient dialysis from 0.15 to 0.05 M $CaCl_2$ resulted in no observable changes in the circular dichroism spectra of DNA or histone IV, the spectrum of nucleohistone IV underwent major changes suggestive of conformational alteration in both the DNA and protein components of the nucleohistone (Figure 3). The region of the circular dichroism spectrum above 240 nm is markedly different from DNA in either 0.001 M Tris (pH 7.0) or 0.05 M $CaCl_2$–0.001 M Tris (pH 7.0). The 275-nm band of the DNA component of nucleohistone IV is slightly blue shifted and enhanced in amplitude ($[\theta]_{272}$ 10,300) while the negative 245-nm DNA band is concurrently decreased and blue shifted to 243 nm ($[\theta]_{243}$ −6300). A crossover point is observed at 254 nm. The region of the spectrum below 240 nm is characterized by a slight negative shoulder at approximately 230 nm and a positive shoulder at about 215 nm. Except for the absence of a negative band at 305 nm the circular dichroism spectrum of nucleohistone IV at 0.05 M $CaCl_2$ (Figure 3) is remarkably similar to the spectrum observed by Shih and Fasman (1971) for a DNA:histone IV complex under similar ionic strength conditions (0.14 M NaF).

The circular dichroism spectrum of nucleohistone IV at 0.005 M $CaCl_2$ (Figure 4) is markedly different from either the spectrum of nucleohistone IV at 0.05 or 0.15 M $CaCl_2$. The positive DNA circular dichroism band at 275 nm is diminished in magnitude compared to both DNA in 0.005 M $CaCl_2$ and in 0.001 M Tris and is considerably red shifted ($[\theta]_{284}$ +3200). A crossover point is observed at 265 nm and the negative DNA band at 245 nm is slightly diminished and red shifted ($[\theta]_{248}$ −6900). The region of the circular dichroism spectrum of nucleohistone IV below 240 nm is characterized solely by a small negative band at 208 nm ($[\theta]_{208}$ −2400). This low wavelength region of the spectrum is markedly similar to the same spectral region in the circular dichroism spectrum of a nucleohistone IV complex studied by Li *et al.* (1971) at low ionic strength. The DNA portion of this spectrum, particularly the positive band at 284 nm, is quite similar to the DNA region of the circular dichroism spectrum of isolated chromatin (Tuan and Bonner, 1969; Permogorov *et al.*, 1970; Simpson and Sober, 1970; Shih and Fasman, 1970; Wagner and Spelsberg, 1971).

An Evaluation of Possible Circular Dichroism Artifacts Due to Light Scattering. The three nucleohistone IV preparations

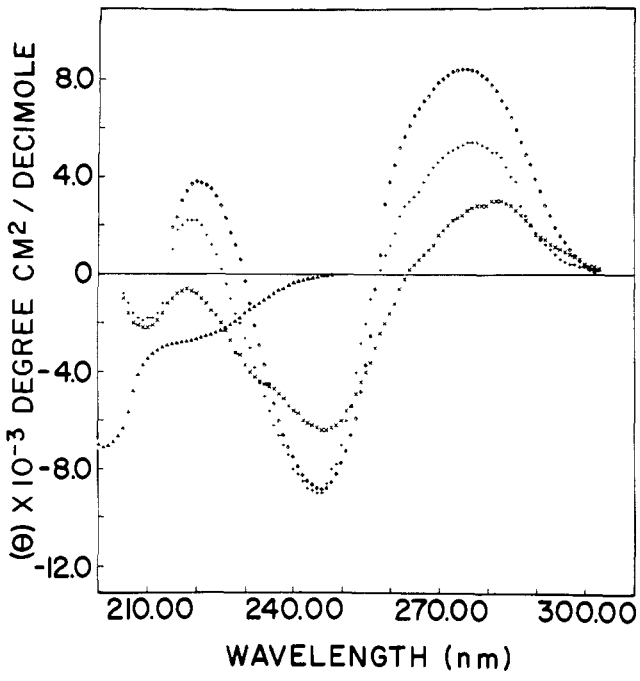

FIGURE 4: Circular dichroism spectra of DNA, histone IV, and nucleohistone IV in 0.005 M $CaCl_2$: (△) histone IV in 0.005 M $CaCl_2$–0.001 M Tris (pH 7.0); (□) DNA in 0.001 M Tris (pH 7.0); (+) DNA in 0.05 M $CaCl_2$–0.001 M Tris (pH 7.0); (×) nucleohistone IV prepared by $CaCl_2$ gradient dialysis in 5 M urea terminated at 0.005 M $CaCl_2$ in 0.005 M $CaCl_2$–0.001 M Tris (pH 7.0).

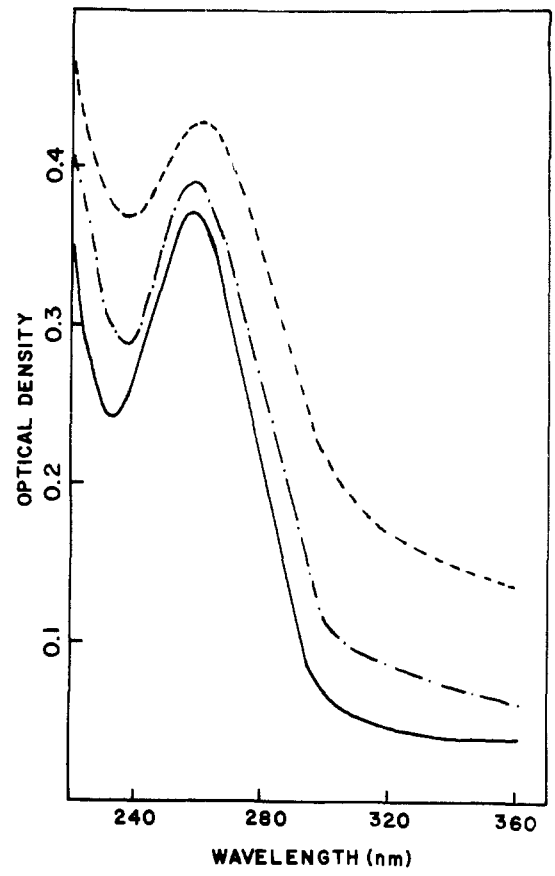

FIGURE 5: Ultraviolet absorption spectra of Ca^{2+} nucleohistone IV. The solution measured are a 10-fold dilution of those used to measure the circular dichroism spectra of Ca^{2+} nucleohistone IV shown in Figures 2, 3, and 4: (—) Ca^{2+} nucleohistone IV in 0.15 M $CaCl_2$–0.001 M Tris (pH 7.0), (—·—) Ca^{2+} nucleohistone IV in 0.05 M $CaCl_2$–0.001 M Tris (pH 7.0), (- - -) Ca^{2+} nucleohistone IV in 0.005 M $CaCl_2$–0.001 M Tris (pH 7.0). The spectra were measured in a 1-cm path-length cell.

studied each displayed some slight turbidity. The turbidity of these solutions was inversely proportional to the $CaCl_2$ concentration as may be seen from the ultraviolet absorption spectra shown in Figure 5. It has been suggested by Urry *et al.* (1970) that certain circular dichroism spectral changes are the result of light-scattering artifacts of turbid solutions. In the present study this explanation seems particularly unlikely for several reasons. (1) The direction of changes in the magnitude of circular dichroism bands are unrelated to changes in turbidity (*i.e.*, the solution of intermediate turbidity (Figure 3) displays an enhanced band at 275 nm while the solution of highest turbidity shows a diminished band at this wavelength), (2) changes in the crossover points of the circular dichroism spectra in question are observed while changes in crossover point due to light scattering have been specifically ruled out by the calculation of Urry *et al.* (1970), and (3) no long-wavelength spectral distortions characteristic of highly scattering samples are observed. On the basis of these and other arguments we have concluded that the spectral changes observed are due to intrinsic alteration in geometric aspects of nucleohistone IV and not to light-scattering phenomenon.

Discussion

Conformational Aspects of the DNA Component of Ca^{2+} Nucleohistone IV. DNA, the primary genetic molecule, has been shown by X-ray diffraction studies to have a characteristic helical structure which may assume several different conformations (A, B, or C form) depending upon the degree of hydration of the macromolecule (Langridge *et al.*, 1960; Marvin *et al.*, 1961; Fuller *et al.*, 1965). The conformation of DNA in aqueous solution has recently been shown to be a slight variation of the highly hydrated B form (Bram, 1971). The circular dichroism spectrum of DNA in aqueous solution is characterized by a positive and negative band on either side of the wavelength of maximum DNA absorption (Brahms and Mommaerts, 1964). This spectrum has been termed conservative (Bush and Brahms, 1967) and is markedly different from the nonconservative spectrum of double-stranded RNA (Brahms and Mommaerts, 1964; Samejima *et al.*, 1968). The theoretical calculations of Tinoco (1964) have suggested that the conservative spectrum of DNA is a result of mutually cancelling interactions between the transition moments and polarizabilities of neighboring bases oriented almost perpendicular to the helix axis. These interactions are also held responsible for the low ellipticities associated with the conservative spectrum. The nonconservative RNA spectrum is seen by Tinoco and coworkers (Tinoco, 1964; Johnson and Tinoco, 1969) to arise from the large degree of tilt with respect to the helix axis displayed by RNA bases.

Circular dichroism studies of DNA in special environments have resulted in nonconservative DNA spectra. Ethylene glycol (95%) solutions of DNA give rise to a negative nonconservative circular dichroism spectrum, while DNA in 80% ethanol yields a positive nonconservative spectrum (Brahms and Mommaerts, 1964). Johnson and Tinoco (1969) indicate that the bases of DNA in these solvents are tilted with respect to the helix axis to a large extent and may well be oriented in the conformation of the A form of DNA. The effect of high

salt concentration on DNA conformation has been studied by Tunis-Schneider and Maestre (1970) by circular dichroism spectroscopy. A negative nonconservative spectrum was observed at 4–6 M LiCl. These workers have also observed similar spectra from films of Li:DNA at 66% relative humidity and suggest that the DNA is in the C form (Tunis-Schneider and Maestre, 1970).

In the study described herein a nucleohistone composed of DNA and histone IV in a 1:2 molar residue ratio has been prepared in the presence of Ca^{2+} and studied at three specific Ca^{2+} concentrations. Three unique circular dichroism spectra (Figures 2–4) in the region of DNA absorption were observed suggesting a surprising plasticity for the DNA component of this nucleohistone. At the highest Ca^{2+} concentration studied (0.15 M), the circular dichroism spectrum of the DNA component of Ca^{2+} nucleohistone IV (Figure 2) is indistinguishable from DNA in 0.15 M $CaCl_2$. This result suggests that a nucleohistone IV complex is present at 0.15 M $CaCl_2$ in which the conformation of the DNA component is independent of the histone component and dependent only upon solvent conditions. Similar results have been observed for other nucleohistone IV complexes in our laboratory (Wagner, 1970) and elsewhere (Li *et al.*, 1971).

The nucleohistone IV prepared at 0.05 M $CaCl_2$ (Figure 3) differs substantially from nucleohistone IV prepared at 0.15 M $CaCl_2$. The circular dichroism spectrum of Ca^{2+} nucleohistone IV (0.05 M $CaCl_2$) (Figure 3) is markedly nonconservative displaying an enhanced positive 275-nm band and a diminished negative 245-nm band. The entire spectrum is quite similar to the circular dichroism spectra recorded by Shih and Fasman (1971) for nucleohistone IV in 0.14 M NaF and by Olins and Olins (1971) for nucleohistone IV prepared from guanidine hydrochloride. As has been pointed out by Shih and Fasman (1971), this DNA spectrum closely resembles the spectrum calculated by Johnson and Tinoco (1969) for A-form DNA and the DNA spectrum measured in 80% ethanol (Brahms and Mommaerts, 1964) and in a film of A-form DNA (Tunis-Schneider and Maestre, 1970). The ionic strength in which this Ca^{2+} nucleohistone IV was studied is similar to the ionic strength of the medium in which Shih and Fasman (1971) studied their DNA:histone IV complex suggesting that solvent ionic strength is an important requirement for this conformation of nucleohistone IV. Although Ca^{2+} nucleohistone IV (0.05 M $CaCl_2$) displays a similar circular dichroism spectrum to Shih and Fasman's DNA:histone IV complex, the spectra are not identical. The absolute magnitude of the positive and negative DNA bands are not the same, probably due to the structural effect of Ca^{2+} on the DNA component of the nucleohistone. The small negative band at 305 nm observed by Shih and Fasman (1971) in the circular dichroism spectrum of DNA:histone IV complexes is not present in our spectra. The absence of this band may be due to the higher histone:DNA ratio in Ca^{2+} nucleohistone IV (increasing the histone:DNA ratio in Shih and Fasman's histone IV:DNA complex resulted in a progressive decrease in the magnitude of the 305-nm band) or to the presence of Ca^{2+}. Whereas in the presence of Na^+, continued dialysis of nucleohistone IV complexes to low ionic strength in 5 M urea resulted in nucleohistones displaying circular dichroism spectra indistinguishable from purified DNA (Shih and Fasman, 1971), in the presence of Ca^{2+} similar dialysis yields a complex [Ca^{2+} nucleohistone IV (0.005 M CaCl)] with a circular dichroism spectrum markedly different from DNA in 0.001 M Tris or in 0.005 M $CaCl_2$ (Figure 4) or from the DNA component of Ca^{2+} nucleohistone IV (0.05 M $CaCl_2$) (Figure 3).

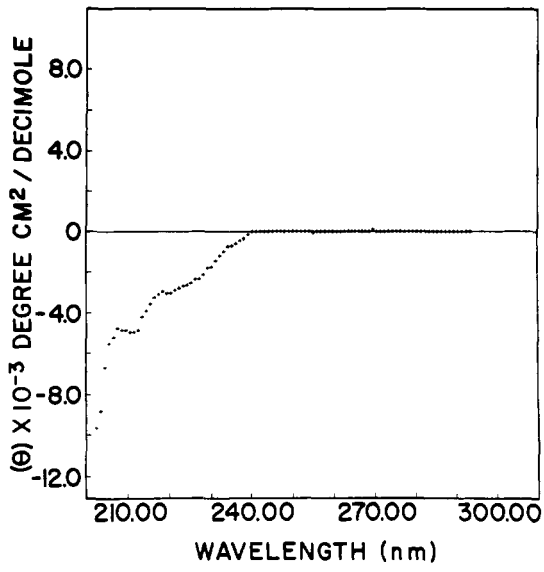

FIGURE 6: Difference circular dichroism spectrum of nucleohistone IV prepared by $CaCl_2$ gradient dialysis in 5 M urea terminated at 0.15 M $CaCl_2$ minus DNA in 0.15 M $CaCl_2$. Solution are buffered in 0.001 M Tris at pH 7.0.

The positive DNA band in the spectrum of Ca^{2+} nucleohistone IV (0.005 M $CaCl_2$) (Figure 4) is characterized by a marked decrease in ellipticity associated with a red shift. The crossover point is shifted from its position in DNA (257 nm) to 265 nm and the negative DNA band is diminished and red shifted to a small extent. On the basis of this circular dichroism spectrum, it appears that the DNA conformation within nucleohistone IV (0.005 M $CaCl_2$) is unique among previously studied nucleohistones containing histone IV or other histone species. The DNA circular dichroism spectrum of this Ca^{2+} nucleohistone IV is markedly similar to that displayed by the DNA component of chromatin. This similarity will be investigated in greater detail later in this discussion.

Conformational Aspects of the Protein Component of Ca^{2+} Nucleohistone IV. Although histone IV conformation has previously been reported (Shih and Fasman, 1971) to undergo substantial changes due to alteration in solvent ionic strength, in our experience the circular dichroism spectrum of histone IV remains substantially the same from 0.15 to 0.005 M $CaCl_2$ (Figures 2–4). The circular dichroism spectra of histone IV in this $CaCl_2$ concentration range are all characteristic of a largely random coil protein. Much like the DNA component of Ca^{2+} nucleohistone IV, histone IV bound to DNA in the presence of Ca^{2+} displays a range of different circular dichroism spectra. Since the DNA region (above 240 nm) of the circular dichroism spectrum of Ca^{2+} nucleohistone IV (0.15 M $CaCl_2$) (Figure 2) was found to be indistinguishable from DNA in 0.15 M $CaCl_2$ the histone IV contribution to this spectrum may be estimated by subtraction of the DNA spectrum from the nucleohistone spectrum. The resulting spectrum (Figure 6) is characteristic of a random coil protein with a minor α-helical contribution.

The low-wavelength regions of the circular dichroism spectra of Ca^{2+} nucleohistone IV both in 0.05 and 0.005 M $CaCl_2$ display little or no negative ellipticity. The low-wavelength region of these spectra are virtually identical to the low-wavelength regions of previously reported nucleohistone IV spectra (Shih and Fasman, 1971; Olins and Olins, 1971; Li *et al.*, 1971). The total absence of negative ellipticity in the low-

wavelength, protein, region of the circular dichroism spectrum of Ca^{2+} nucleohistone IV in 0.05 M $CaCl_2$ (Figure 3) is particularly unusual. This spectral characteristic may be the result of a major change in the conformational aspects of the histone IV backbone or be the result of the emergence of a large positive DNA band in this region of the spectrum offsetting negative protein circular dichroism bands. We agree with Shih and Fasman (1971), who have observed an almost identical spectrum in this wavelength region for a histone IV:DNA complex, that structural analysis on the basis of such an unusual and unexplained protein spectral region is not feasible. This same difficulty is encountered in any attempt to rigorously interpret the low-wavelength region of the circular dichroism spectrum of Ca^{2+} nucleohistone IV in 0.005 M $CaCl_2$.

Ca^{2+} Nucleohistone IV as a Structural Model for Chromatin. To date there have been no apparent similarities between the circular dichroism spectrum of the DNA component of chromatin (Permogorov *et al.*, 1970; Simpson and Sober, 1970; Shih and Fasman, 1970) and the spectrum of the DNA component of simple histone:DNA complexes (Fasman *et al.*, 1970; Olins and Olins, 1971; Shih and Fasman, 1971; Li *et al.*, 1971).

The circular dichroism spectrum of the DNA component of chromatin is characterized by a 275-nm positive band which is slightly red shifted and diminished by about 50% in respect to the 275-nm band of purified DNA and a slightly red-shifted crossover point (Permogorov *et al.*, 1970; Simpson and Sober, 1970; Shih and Fasman, 1970). Although Ca^{2+} nucleohistone IV in 0.005 M $CaCl_2$ does not display the exact spectrum shown by chromatin, the spectral differences between DNA in 0.005 M $CaCl_2$ and Ca^{2+} nucleohistone IV in 0.005 M $CaCl_2$ (Figure 4) are almost identical to the difference observed between DNA and the DNA component of chromatin. This observation suggests that histone IV may have the same conformational effects on DNA in Ca^{2+} nucleohistone IV that the protein components of chromatin exert upon the DNA component of this isolated genetic structure. This result is not particularly surprising in view of recent work indicating that histone IV may have a major DNA conformation determining role in chromatin (Wagner and Spelsberg, 1971).

Although five detailed studies of histone IV:DNA complexes (Wagner, 1970; Shih and Fasman, 1971; Olins and Olins, 1971; Li *et al.*, 1971) have been carried out during the past several years, only Ca^{2+} nucleohistone IV displayed circular dichroism spectral characteristics which model chromatin. This result points out the crucial role of Ca^{2+} ion in the structure of Ca^{2+} nucleohistone IV and suggests the possibility of a structural role for divalent metal ions in chromosomal material.

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