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Circular Dichroism of Calf Liver Nucleohistone*

Robert T. Simpson and Herbert A. Sober

ABSTRACT: The circular dichroism of calf liver soluble nucleohistone, its components, and its complexes with a number of small molecules has been investigated in an attempt to probe the conformation of nucleic acids and proteins in chromatin. The circular dichroism of nucleohistone is complex, with a negative band at 300 nm (θ = −400 deg cm²/dmole), positive bands at 280 (+5500) and 272 (+6000) nm, and negative bands at 245 (~−6000), 223 (−11,500), and 207 (−13,500) nm, with molecular ellipticities based on the concentration of nucleotide bases for the first four, and on the concentration of protein residues for the last two. The magnitude of the main positive circular dichroism band associated with DNA in nucleohistone is reduced by about 30% from that of the same DNA alone in solution, consistent with partial base tilting or unstacking on formation of a superhelical-coiled structure for chromatin. When compared with helical synthetic polyamino acids the proteins of chromatin appear to exist in about 35–40% α-helical conformation. Experiments with the components of soluble nucleohistone suggest that splitting in the major positive circular dichroism band might arise from the contributions of both DNA and chromosomal RNA. The altered conformation state of DNA in nucleohistones is the result of constraints imposed by the presence of histone, primarily the slightly lysine-rich fraction. Thus, removal of this histone fraction by controlled salt extraction leads to a major alteration in the circular dichroism properties of nucleohistone, while removal of very lysine-rich or arginine-rich histones have little additional effect. Addition of calcium or mercuric ion to either nucleohistone or to isolated DNA leads to similar circular dichroism changes for both complexed and free DNA, suggesting that the conformation of the nucleic acid moiety is similar in these two cases insofar as it is reflected by these probes. In contrast, different circular dichroism patterns are obtained for the complexes of acridine orange with DNA or nucleohistone.

The spectral changes obtained upon the addition of acridine orange to nucleohistone are more characteristic of the interaction of this dye with helical polypeptides than with helical DNA.

Chromatin, the interphase form of the chromosomes of the cell nucleus, consists of DNA, a small amount of RNA, and proteins, both histones and acidic proteins (*cf.* Bonner *et al.*, 1968a). Detailed knowledge of the conformational properties of these complexed moieties might shed light on the mechanism of suppression of a large part of the genetic information in differentiated eukaryotic cells. Similarly, the selective expression of that information appropriate to the function of a particular cell may well be determined by the structure of the chromatin. Carefully isolated chromatin seems to be similar to the material present *in vivo* in the cell nucleus, by the criterion of support of DNA-dependent RNA synthesis and the nature of the RNA thus formed (Bonner *et al.*, 1963; Paul and Gilmour, 1968; Smith *et al.*, 1969). Shearing chromatin to produce "soluble nucleohistone"[1] appears to produce little alteration in its biological properties. The physicochemical and biological properties of soluble nucleohistone, however, differ significantly from those of its component DNA and proteins.

The conformation of DNA and protein in nucleohistone has been examined by infrared spectrophotometry (Zubay and Doty, 1959), optical rotatory dispersion (Zubay and Doty, 1959; Oriel, 1966), ultraviolet spectrophotometry (Bonner and Tuan, 1968), melting studies (Ohlenbusch *et al.*, 1967),

* From the Laboratory of Nutrition and Endocrinology, National Institute of Arthritis and Metabolic Diseases, National Institutes of Health, Public Health Service, U. S. Department of Health, Education, and Welfare, Bethesda, Maryland 20014. *Received March 4, 1970.*

[1] In agreement with general usage the term nucleohistone will be employed to describe sheared chromatin, although such chromatin contains a more complex mixture of species than is suggested by this descriptive name.

flow birefringence and dichroism (Ohba, 1966), hydrogen–deuterium exchange (Bradbury and Crane-Robinson, 1964), and X-ray diffraction (Wilkins *et al.*, 1959). Two other techniques available for the conformational examination of nucleic acids and proteins, *i.e.*, circular dichroism and fluorescence, have apparently not yet been applied to investigation of the structure of nucleohistone. These latter two techniques provide even more information when small molecules are used as probes for the conformational properties of either nucleic acids or proteins. The present report describes the circular dichroism spectra of soluble nucleohistone, the effects of fractional removal of certain protein components, and interaction with various chemical species which form chemooptical probes for the conformation of DNA or protein.

Experimental Section

Chromatin was isolated from purified nuclei of calf liver. Livers were obtained immediately after death of the animal and were either cooled in ice and processed within 2 hr (procedure 1) or immediately frozen in Dry Ice and stored at −100°F until use, a period of less than 10 days (procedure 2).

Procedure 1. Nuclei were isolated using Triton X-100 as suggested by Hymer and Kuff (1964). The perfused, trimmed liver was minced in a commercial meat grinder. The mince was homogenized as a 10% (w/v) suspension in 3-l. lots in a Waring Blendor for 4 min at 50 V in 250 mM sucrose–3 mM $CaCl_2$. The pooled homogenate was strained through four layers of fine mesh gauze and nuclei were isolated by centrifugation for 10 min at 1000*g* in an International PR-6 centrifuge. The crude nuclear fraction was suspended in 250 mM sucrose–1 mM $MgCl_2$–0.05 M Tris·HCl (pH 8.0) using 1 ml of buffer/g of liver. This suspension was homogenized at 32–37 V in the Waring Blendor and Triton X-100 was added to a final concentration of 1% (v/v) over a period of 2 min. Nuclei were then sedimented from the homogenate by centrifugation at 1000*g* for 10 min. Homogenization and sedimentation were repeated until the pellet was colorless and the supernatant was clear, usually requiring two–three cycles. The final pellet was suspended in a small volume of 250 mM sucrose–3 mM $CaCl_2$ and stored at 2–4°. Nuclei obtained by this procedure were round, with well-preserved nucleoli, and lacked cytoplasmic contamination when examined either by phase contrast microscopy or by light microscopy following staining with Giemsa stain. Little amorphous material was present, and the proportion of whole cells to nuclei was less than $^1/_{100}$. The average content of these nuclei was 7.1 pg of DNA and 28 pg of protein per nucleus.

Procedure 2. Nuclei were prepared from frozen liver using methodology initially established by Schneider and Petermann (1950), as modified by Allfrey and coworkers (Allfrey and Mirsky, 1957; Allfrey *et al.*, 1964) and recently described by Wang (1968). Homogenization and washing were carried out in ten volumes of 250 mM sucrose–3 mM $CaCl_2$, and the purified nuclei were then sedimented through a Ficoll (Pharmacia Corp.) density barrier (Wang, 1968). Such nuclei were as pure as those prepared by procedure 1 in terms of lack of whole cell or cytoplasmic contamination, but contained a considerable amount of amorphous material, presumably chromatin resulting from lysis of nuclei. This is reflected in the higher apparent DNA content per nucleus, 12.7 pg, found in these preparations. The ratio of protein to DNA was similar to that of nuclei isolated by the use of the detergent. No differences were noted in the chromatins prepared from nuclei obtained by the two different procedures.

Chromatin was isolated from nuclei as described by Huang and Huang (1969). Nuclei were homogenized in and sedimented twice from 75 mM NaCl–24 mM EDTA (pH 8.0) and then homogenized in and sedimented from media of decreasing ionic strength (0.05–0.001 M Tris·HCl) at pH 8.0. The isolated chromatin was sheared as described by Bonner *et al.* (1968b) and sedimented at 10,000*g* for 30 min to remove unsheared material. Soluble nucleohistone thus prepared was a slightly opalescent material with a ratio of $A_{320}:A_{260}$ of less than 0.04 and a mass ratio of protein to DNA of 2.0.

DNA was isolated from unsheared chromatin by dissolving chromatin at a DNA concentration of less than 1 mg/ml in 2 M NaCl–0.005 M Tris·HCl (pH 8.0), a condition which has been shown to dissociate DNA from histones and chromosomal RNA (Bekhor *et al.*, 1969). The solution was then applied to a column of Sepharose 4B (Pharmacia Corp.) of 20 times the sample volume and having a length to diameter ratio of 20, and eluted with 2 M NaCl at a flow rate of 4–5 ml/hr cm^2. DNA emerged in the void volume under such conditions of gel filtration, while the proteins and RNA of chromatin were eluted with the small molecules at the salt boundary. DNA isolated by this procedure contained less than 2% protein, and was $>20 \times 10^6$ in molecular weight, estimated by gel filtration.

DNA concentrations were determined by reaction with diphenylamine as described by Schneider (1957) using calf thymus DNA (Mann Corp.) as a standard. Protein contents were measured by the Lowry *et al.* (1951) reaction as described by Layne (1957) with crystalline bovine albumin as standard.

Absorbance measurements at discrete wavelengths were obtained with a Zeiss PMQ II spectrophotometer. Continuous absorption spectra were obtained with a Beckman DB-G instrument equipped with a 10-in. linear-log recorder. pH measurements were made with a Corning Model 12 pH meter equipped with a general purpose glass electrode. The pH values listed are those of the various solutions at 25°.

Circular dichroism spectra were obtained with the Cary Model 60 spectropolarimeter equipped with a Cary Model 6002 circular dishroism attachment. The instrument was standardized at least weekly with a solution of *d*-10-camphorsulfonic acid (1 mg/ml in water), and was checked for absorption artefacts by recording the spectrum of the standard in concert with that of an optically inactive substance (usually potassium dichromate) sufficient to give an absorbance of over 2 at the wavelength of maximum ellipticity. All spectra were recorded with the slit program of the instrument adjusted to a spectral band width of 15 Å. For routine measurements, circular dichroism spectra were recorded at a DNA concentration of 0.050 mg/ml in a 1-cm cell over the region from 250 to 350 nm, and a 0.20-cm path-length cell in the region from 200 to 250 nm. Ellipticities are reported as mole ellipticities, with the dimensions of deg cm^2/dmole, except where noted.

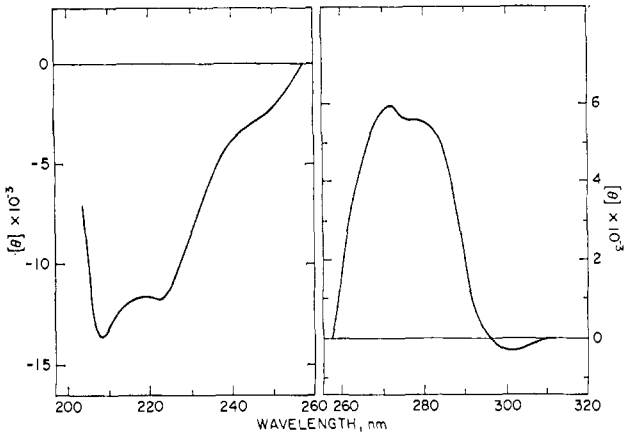

FIGURE 1: Circular dichroism spectrum of soluble nucleohistone. The spectrum was recorded at 28° using a solution containing 0.05 mg/ml of DNA with a cell of path length 1.00 cm for the wavelength range from 320 to 250 nm, and of path length 0.20 cm for the wavelength range from 260 to 200 nm. The buffer was 0.001 M Tris·acetate (pH 7.8). Data are molecular ellipticities based on the concentration of DNA bases >250 nm; or protein residues, <250 nm.

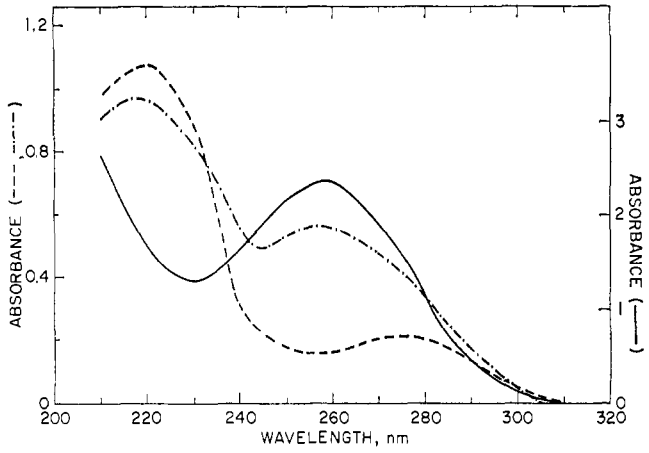

FIGURE 2: Absorption spectra of the components of soluble nucleohistone. All spectra were obtained in 0.05 M Tris·acetate (pH 8.0). The samples were adjusted to be of equal content in terms of dilution from the original sample of nucleohistone applied to the Sepharose column (see Experimental Section), assuming total recovery of all material added to the column. (——) DNA fraction, (----) retarded fraction containing only protein, soluble in 0.1 M NaCl, and (·–·–·–) retarded fraction containing both protein and RNA, soluble in 1.0 M NaCl.

Results

Several optically active absorption bands were obtained for soluble nucleohistone (Figure 1). For convenience, the circular dichroism spectrum can be divided into two regions, above and below 250 nm, respectively. In the region from 250 to 320 nm, the spectrum would be expected to arise from absorption due to the nucleic acids of nucleohistone. The data in this wavelength range are reported as mole ellipticities based upon the concentration of nucleotide bases present. Below 250 nm, the contributions of the nucleic acids become relatively small when compared with those of the protein polypeptide backbone. The spectral data in this region are reported as mole ellipticities based upon the concentration of protein amino acid residues in the sample under investigation.

At least three optically active bands are present in nucleohistone in the longer wavelength region (Figure 1). A small negative band is present at about 300 nm, with a maximum ellipticity of about −400. This band is similar in location and sign to that observed for DNA by Sarker *et al.* (1967) but is of greater magnitude. The main positive ellipticity band above 260 nm is apparently split into two components, with positive maxima of +5500 and +6000 present at 280 and 272 nm, respectively. In the lower wavelength region, a small negative band is apparent at about 245 nm, as expected from previous studies of the circular dichroism of DNA (Brahms and Mommaerts, 1964; Sarker *et al.*, 1967; Mommaerts, 1968; also *vide infra*), although it is difficult to dissociate this band from those associated with protein. Well-defined negative maxima are present at 207 and 223 nm, as expected for a protein with some degree of organized α-helical structure. The magnitude of the ellipticity at these wavelengths is −11,500 and −13,500, respectively. When compared with synthetic polyamino acids in mixed random and α-helical conformation (Beychok, 1968; Greenfield and Fasman, 1969), this corresponds to about 35–40% of α helix, somewhat lower than that estimated for nucleohistones (Zubay and Doty, 1959) from visible optical rotatory dispersion.

These spectral features of nucleohistone at low ionic strength do not appear to arise as a consequence of aggregation or disaggregation of the chemical species under study, since spectra recorded at a 20-fold higher concentration of nucleohistone, in an appropriately shorter path-length cell, were identical with those reported above. Similarly, the use of shearing to reduce the size of chromatin apparently does not influence the circular dichroism properties of the species since the same circular dichroism spectrum is obtained when calf liver nuclei are gently lysed with minimal shearing by suspending them in a medium of low ionic strength in a Pasteur pipet. These results do not, of course, preclude the occurrence of a different conformation for DNA or proteins when present in nuclei.

The circular dichroism spectra of the components of nucleohistone were examined to ascertain whether the splitting observed for the complex species in the 250–320-nm region might be associated with the presence of the known components of nucleohistone. As noted above, when nucleohistone is chromatographed in 2 M NaCl on Sepharose 4B, DNA emerges as a clearly defined peak at the void volume, while a second well-separated peak contains both protein and RNA. When this second peak is dialyzed to distilled water and lyophilized, and then dissolved in media of various ionic strengths, a further partition of the components of chromatin is obtained. At pH 8.0, in 0.05 M Tris·acetate, one fraction dissolves in 0.1 M NaCl, while a second fraction dissolves only when the ionic strength is increased to 1.0 with NaCl. The material soluble in 0.1 M NaCl is entirely protein in nature, while the material soluble in 1.0 M NaCl contains both protein and RNA, in an approximately 7:1 mass ratio.

The absorption spectra of these three species are presented in Figure 2, and the corresponding circular dichroism spectra

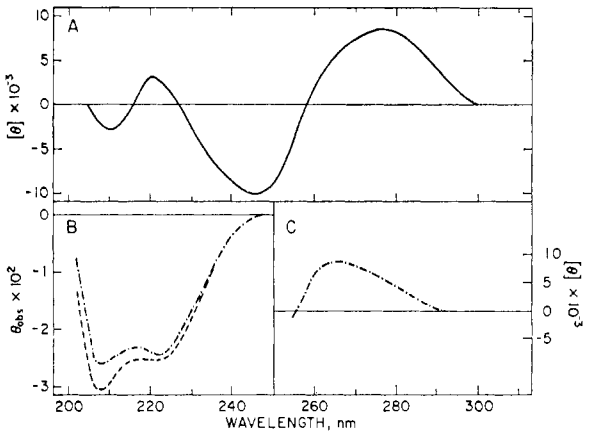

FIGURE 3: Circular dichroism spectra of the components of soluble nucleohistone. Symbols are as in Figure 2. (A) The circular dichroism of DNA-containing fractions. (B) Circular dichroism of the retarded fractions in the region of peptide-bond absorption. Ellipticities are observed values for the solutions whose absorption spectra are reported in Figure 2. (C) Circular dichroism of retarded fractions in the region of absorption of the nucleic acids.

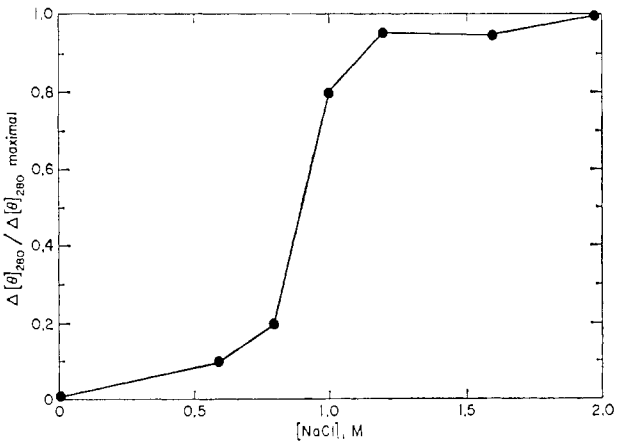

FIGURE 4: Effect of increasing ionic strength with NaCl on the ellipticity of nucleohistone at 280 nm. The change in molecular ellipticity, plotted as the fraction of the maximal observed change, is plotted as a function of the molarity of NaCl in the sample. All data obtained in 0.005 M Tris·acetate (pH 7.8).

in Figure 3. The ultraviolet absorption properties of these fractions are consistent with the analytical data. Thus, the DNA fraction has a peak at 260 nm alone. The fraction consisting only of protein has absorption maxima at 275 and 220 nm, while that which contains both protein and RNA has maxima at 260 and 220 nm. The circular dichroism spectrum of the DNA is quite consistent with those reported by others (Brahms and Mommaerts, 1964; Sarkar *et al.*, 1967; Mommaerts, 1968). Positive ellipticity maxima are present at 276 and 220 nm, while negative maxima are located at 246 and 210 nm. The molecular ellipticities of the longer wavelength maxima are +7,800 and −10,500, while those of the lower wavelength maxima are ±2,500 (Figure 3A). Both of the retarded fractions exhibit circular dichroism spectra at short wavelengths which are consistent with a moderate degree of α helix in the protein components (Figure 3B). Only the fraction which contains RNA, however, has an ellipticity band at wavelengths longer than 250 nm showing a positive band with a molecular ellipticity of about +8000 at 265 nm (Figure 3C). No corresponding negative band at lower wavelengths was observed in these experiments, consistent with the circular dichroism patterns of other RNA species, which generally exhibit nonconservative circular dichroism spectra (Busch and Brahms, 1967; Sarkar *et al.*, 1967; Mommaerts, 1968; Johnson and Tinoco, 1969). Confirmation of this observation must wait the isolation of species which contain less protein to enable more accurate observation of the spectral region below 260 nm. It is noteworthy, however, that the two positive bands at 272 and 280 nm (Figure 1), which occur in native isolated nucleohistone, may be accounted for by the nucleic acid components of this complex species.

The role of various histone fractions in maintaining the conformation of DNA in nucleohistone was examined by fractional removal of these proteins with NaCl. Increasing ionic strength with either NaCl or $NaClO_4$ has previously been shown to dissociate the various histone fractions of nucleohistone in a specific order (Ohba, 1965; Ohlenbusch *et al.*, 1967). First, the very lysine-rich histones were removed by 0.6 M NaCl, followed by the slightly lysine-rich at 0.6–1.2 M NaCl, and finally the arginine-rich group at 1.2–2.0 M NaCl. When the circular dichroism spectra of nucleohistone are examined in 2 M NaCl, a salt concentration sufficient to dissociate histones from DNA, an increased ellipticity of about 30% at the positive long-wavelength maximum is noted. This increase develops over a range of 0.6–1.2 M NaCl, with little alteration occurring at either higher or lower concentrations of the salt (Figure 4). The salt concentration which leads to the increase in ellipticity of the DNA of these partially deproteinized nucleohistones is that associated with the removal of the slightly lysine-rich histones (Ohba, 1965; Ohlenbusch *et al.*, 1967). The doublet band observed for nucleohistone persists in the totally dissociated species.

A number of small molecules interact with DNA in such a manner as to alter the circular dichroism or optical rotatory dispersion properties of the macromolecule itself or alternatively, to generate a new chromophore with properties which reflect the conformation of the DNA (Neville and Bradley, 1961; Cheng, 1965; Blake and Peacocke, 1965; Gabbay and Mitschele, 1969; Aktipis and Martz, 1969). Several such agents were examined in terms of their effects on the circular dichroism spectra of nucleohistone on the one hand, and the DNA derived from that nucleohistone, on the other.

Several divalent cations, such as calcium, led to a diminution of the Cotton effect centered at about 265 nm, when added in a twofold molar excess to DNA (Cheng, 1965). Calcium, at a molar concentration of three times that of the DNA bases, leads to a 15% decrease in the magnitude of the positive ellipticity band at 276 nm for calf liver DNA and a 25% decrease for nucleohistone. Calcium has no effect on the circular dichroism spectra at wavelengths less than 250 nm.

Mercuric ion induces much more striking alterations in the circular dichroism spectrum of DNA, both when free and when complexed with proteins in chromatin. In both cases, the spectrum is altered so as to create negative maxima,

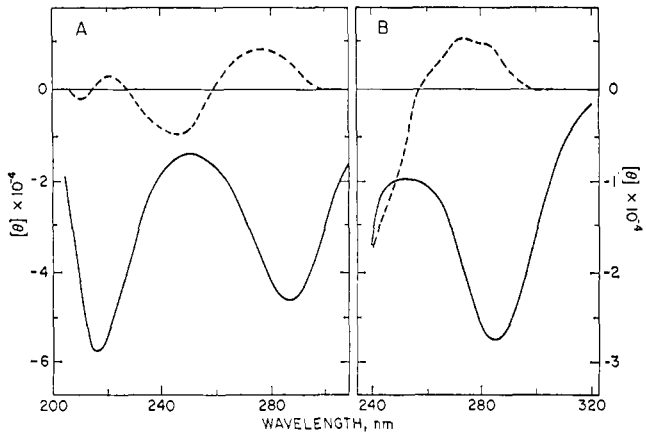

FIGURE 5: Circular dichroism spectra of DNA (A) and nucleohistone (B) in the presence (——) and absence (----) of 1×10^{-4} M $HgCl_2$. Spectra were obtained in 0.001 M Tris·acetate (pH 7.8) at a nucleic acid concentration of 0.050 mg/ml.

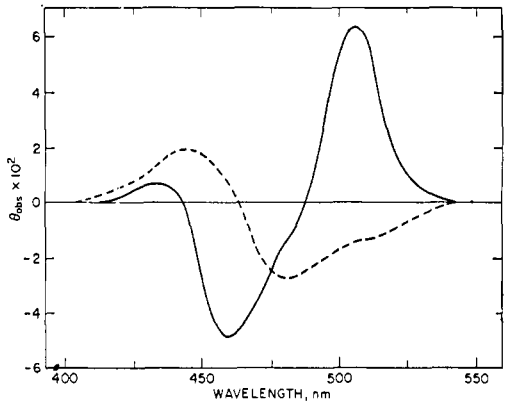

FIGURE 6: Circular dichroism spectra of the complex of acridine orange with DNA (----) and with nucleohistone (——), obtained at 1×10^{-4} M concentrations of both dye and nucleotide bases. The data are reported as the observed ellipticities for these solutions in a 1.00-cm cell. Buffer employed was 0.01 M *N*-2-hydroxyethylpiperazinesulfonic acid (pH 6.9).

with ellipticities five to seven times greater than the largest ellipticity observed for DNA itself in the absence of this cation (Figure 5). Again, little difference is observed between the effects of the agent on DNA free in solution and DNA complexed in nucleohistone. When corrected for the alterations in the circular dichroism spectrum which accompany the interaction of DNA with mercury, there appears to be little effect of the addition of this divalent cation on the circular dichroism properties of the proteins of chromatin. This finding suggests that the proteins of chromatin have not been dissociated from DNA by Hg, nor significantly altered in their interaction with the nucleic acid, since histones are of very low helical content when uncomplexed in solutions of low ionic strength (Bradbury *et al.*, 1965; Jirgensons and Hnilica, 1965; Oh and Blout, 1969). In the case of both free DNA and nucleohistone, the main absorption band of the mercury–nucleic acid complex is red shifted by about 10 nm relative to the free nucleic acid.

Quite dissimilar results are obtained for the free and complexed nucleic acid when acridine orange is utilized as a probe. This symmetric dye lacks optical activity itself, but undergoes spectral shifts and becomes optically active on interaction with either helical polypeptides (Stryer and Blout, 1961; Yamaoka and Resnik, 1966) or helical double-stranded DNA (Neville and Bradley, 1961; Yamaoka and Resnik, 1966). The circular dichroism spectra resulting from interaction of acridine orange with DNA and nucleohistone, at equimolar concentrations of dye and DNA nucleotides, are shown in Figure 6. The asymmetric Cotton effect centered at about 465 nm, previously observed for DNA plus the dye by optical rotatory dispersion (Neville and Bradley, 1961), is seen to be composed of at least two dichroic bands, a negative band centered at 480 nm and a positive band at 445 nm. There is also a shoulder apparent at longer wavelength, 510 nm.

When nucleohistone and dye are examined, a large positive ellipticity band is seen at 505 nm, and a large negative band is located at 460 nm. There is a shoulder at 475 nm and a small positive band at about 430 nm, the magnitude of which depends on exact conditions of dye:DNA concentrations. The pattern of long-wavelength positive, short-wavelength negative ellipticity bands may also be discerned in the complex Cotton effect seen at moderate (~0.2) ionic strength upon interaction of this dye with partially helical poly-L-glutamic acid (Stryer and Blout, 1961) or in studies at much lower dye:residue ratios (Yamaoka and Resnik, 1966).

Discussion

There are two main lines of investigation available for the examination of the structure of complex biological macromolecules: first. X-ray diffraction studies, probably the most potent means of direct structural evaluation, but limited to the crystalline or semicrystalline state, and second, the various physicochemical methods which reflect the conformation of molecules in solution. X-Ray diffraction measurements of nucleohistones have been of limited success in determination of structure, when compared with their successes with DNA and proteins. However, under certain conditions, the characteristic diffraction pattern of double-helical DNA is recognizable in nucleohistone (Wilkins *et al.*, 1959). Other results have suggested a supercoiling of nucleohistone with a pitch of 110 Å (Wilkins *et al.*, 1959; Bonner and Tuan, 1968). However, the conformation of protein in nucleohistone has not yielded to X-ray analysis.

Studies of the conformation of proteins and DNA in nucleohistone by the methods applicable to macromolecules in solution have been rather limited. Zubay and Doty (1959) concluded, on the basis of infrared and visible optical rotatory dispersion studies, that the protein components of nucleohistone were about 50% α-helical while Oriel suggested an α-helical content of only 18% (1966). Using hydrogen–deuterium exchange, Bradbury and Crane-Robinson (1964) obtained a figure of about 60% for the helical content of the proteins of nucleohistone. Other approaches to the conformation of DNA in nucleohistone have been measurement of the hyperchromicity (relative to free DNA) of the DNA of the chromatin (Bonner and Tuan, 1968), measurement of the melting temperature of DNA in intact and deproteinized nucleohistone (Ohlenbusch *et al.*, 1967), and

examination of the flow birefringence and dichroism of nucleohistone and DNA (Ohba, 1966). Briefly, these studies indicated that DNA complexed with histones is shortened, with a partial unstacking of the bases, and some disorientation of the base pairs from their positions perpendicular to the long axis of the molecule. Some of the most potent methods of evaluation of conformation of molecules in solution, *e.g.*, circular dichroism and fluorescence, especially studies of energy transfer, have apparently not yet been applied to nucleohistone. Circular dichroism, the approach utilized in the present investigation of the conformation of nucleohistone, is particularly advantageous in that the individual contributions of proteins and nucleic acids to the observed circular dichroism spectrum are relatively easily separable due to differences in the wavelength of their absorption.

In the absorption region of the nucleic acid components of chromatin, the circular dichroism spectrum of nucleohistone is complex, and appears to be associated with both DNA, and a smaller complex of protein and RNA. Whether this apparent complex is identical with the chromosomal RNA–protein complex described by Bonner and collaborators remains to be established (Huang and Bonner, 1965; Huang, 1967; Huang and Huang, 1969). Further, the ellipticity of the complexed DNA is reduced in magnitude by about 30% from that of free DNA in solution. Such a finding is consistent with a reduction in the perfection of the helix brought about by partial base unstacking, previously postulated to be associated with the supercoiled model of nucleohistone (Bonner and Tuan, 1968). The protein components of nucleohistone appear to be 35–40% α helical, a somewhat lower estimate than that obtained by other methods. There is no evidence for the occurrence of large amounts of β structure in nucleohistone.

The effect of selective dissociation of histones by increasing ionic strength on the circular dichroism spectrum of nucleohistone clearly demonstrates that these proteins are involved in constraining the DNA helix into the form which it occupies in chromatin. Increasing ionic strength from 0.1 to 2.0 with sodium chloride has no detectable effect on the circular dichroism properties of DNA when alone in solution. In contrast, the circular dichroism spectrum of DNA in nucleohistone changes quite abruptly to that of free DNA in solution as the slightly lysine-rich histones are removed at a salt concentration of 0.6–1.2 M NaCl. A broad transition in the properties of DNA was noted when histones were removed in studies of melting temperature (Ohlenbusch *et al.*, 1967) and hyperchromicity (Bonner and Tuan, 1968). Both studies also indicated that the main histone fraction affecting the conformation of DNA in chromatin was the slightly lysine-rich group. In contrast, studies of flow dichroism and birefringence by Ohba (1966), which also demonstrated differences between free DNA and DNA in nucleohistone, attributed these differences primarily to the presence of the arginine-rich histones. These latter measurements are hydrodynamic in character, and thus might reflect processes other than relatively short range, base–base interactions.

A further refinement in the study of macromolecular conformation by spectroscopic methods lies in the utilization of so-called chemooptical probes. Although not covalently incorporated into the molecule, the probing species used in the present investigation all lead to characteristic alterations in the circular dichroism properties of isolated double-stranded helical DNA. Hence, the presence or absence of similar alterations when these species interact with DNA in nucleohistone should allow interpretations bearing on the conformation of DNA in the two conditions, free and complexed.

Calcium and mercury lead to strikingly different effects on the circular dichroism spectra of nucleic acids, but the effect of either agent is nearly the same for either free DNA or DNA complexed in nucleohistone. With both metals there is little if any alteration in the circular dichroism spectrum associated with the protein components of nucleohistone, suggesting that neither agent leads to dissociation of histones from the nucleohistone complex. Thus, it may be inferred that both metals affect the circular dichroism spectra through interaction with the nucleic acid moiety alone. Calcium might have been expected to interact preferentially with the phosphate oxygens of the nucleic acid, having little affinity for nitrogenous ligands (Shack and Bynum, 1959). However, current concepts of the structure of nucleohistone would suggest that the phosphates masked by histones should be relatively inaccessible to solvents.

It has been known for some time that mercury has a profound effect on DNA leading to a completely reversible structural change associated with a marked decrease in viscosity (Katz, 1952), a shift in absorption spectrum (Yamane and Davidson, 1961), and alterations in the optical rotatory dispersion spectrum (Cheng, 1965). On the basis of studies with DNA and model polydeoxyribonucleotides (Yamane and Davidson, 1961), and deoxyribonucleosides (Simpson, 1964), it seems likely that mercury binds between adjacent base pairs, leading to a partial disruption of the helical structure of the nucleic acid. In its reaction with nucleohistone, mercury is apparently small enough to gain entry into the nucleohistone complex but without dissociating its DNA and protein components. The observation that small ions such as mercury can enter the nucleohistone complex and alter its structure significantly, yet without apparent dissociation, may be of related interest in the structural alterations which attend DNA translation and replication.

In contrast to the results obtained with the metals as probes, the use of a larger, organic molecule led to gross differences between nucleohistone and free DNA. A definitive explanation for the Cotton effects which appear on interaction of acridine orange with polyamino acids or nucleic acids is not yet available although a number of alternative hypotheses have been considered (Stryer and Blout, 1961; Neville and Bradley, 1961; Yamaoka and Resnik, 1966). Irrespective of which mechanism applies for the generation of the Cotton effects, certain patterns were apparent with the acridine orange effect in these studies. DNA, at nucleotide to dye ratios of 1:1, led to complex Cotton effects in the region of 465 nm (Neville and Bradley, 1961). These Cotton effects are quite consistent with the ellipticity bands observed for interaction of acridine orange with free DNA in the present studies (Figure 6). In contrast, the positions and signs of the ellipticity bands observed when nucleohistone interacted with the dye are similar to those expected from the optical rotatory dispersion studies of Stryer and Blout (1961) and Yamaoka and Resnik (1966) on acridine orange–poly-L-glutamic acid complexes at moderate ionic strength. These findings would suggest that the probing dye interacts with nucleohistone in a fashion similar to a helical polypep-

tide, implying binding to helical segments of histone surrounding the DNA double helix.

Acknowledgments

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Addendum

While the present results were being prepared for publication, a report by Tuan and Bonner (1969) appeared concerning the optical rotatory dispersion spectra of nucleohistone, histones, and partially deproteinized nucleohistone. The results of that study, concerning the asymmetry of DNA in nucleohistone, and its alterations due to the presence of the various histone fractions, are in agreement with the present study, although the higher resolving power of circular dichroism (*vs.* optical rotatory dispersion) has provided more detailed information.

Shih and Fasman (1970) did observe reduced ellipticity of DNA in calf thymus nucleohistone (personal communication). Permogorov *et al.* (1970) also reported essentially the same decreased ellipticity in calf thymus nucleohistone but noted, however, that the circular dichroism pattern of DNA alone in 2 M NaCl was closely similar to that in nucleohistone. This last observation, which contrasts with results reported in the present study, may have been due to differences in method, reagents or tissue of origin.

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