

Circular dichroism analysis of mononucleosome DNA conformation

(chromatin/nucleosomes)

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ABSTRACT Mononucleosomes were isolated from micrococcal nuclease digests of chicken erythrocyte nuclei. The circular dichroism properties of mononucleosome preparations, differing in average DNA length and in H1 and H5 content, demonstrate that the spectrum of chromatin is due only to the complete structure of its repeating subunits. The nucleoprotein spectra are all altered relative to protein-free DNA by the emergence of a single negative band at 275 nm, similar to the band observed for Ψ DNA. The intensity of the Ψ -type band depends on the proportion of DNA condensed in a specific manner. The Ψ -type band is proposed to be due to the compact DNA tertiary structure; i.e., the manner in which the DNA is wound around the histone core allowing interactions between adjacent turns of the superhelix. This interpretation attributes changes and variability in nucleoprotein circular dichroism spectra under different experimental conditions to alterations in DNA tertiary structure rather than secondary structure.

Evidence has been accumulated that indicates that the folding of DNA in chromatin involves several levels of organization. First, DNA is condensed by a histone octamer into compact subunits, or nucleosome cores. The cores contain approximately 140 base pairs of DNA and two each of the histones H2A, H2B, H3, and H4 (for reviews see refs. 1 and 2). The cores are connected by "linker" DNA regions, the lengths of which are variable and with which the very lysine-rich histone H1 (and H5 in avian erythrocytes) interacts. This level of DNA organization corresponds to the "beads-on-a-string" structure (3). The second level of organization is the 100-Å nucleofilament, in which adjacent nucleosome cores are presumably in contact, and the linker DNA is condensed (3-5). The third level of organization is the 250 to 300-Å diameter chromatin fiber, proposed to be a solenoidal arrangement of the 100-Å nucleofilament (4-7). The second and third organizational levels depend on the presence of H1 and/or salts (4-6).

We have been interested in the possibility that the circular dichroism (CD) spectrum of DNA is sensitive not only to changes in secondary structure (base tilt, twist, etc.), but also to the higher levels of DNA organization in chromatin (8, 9). In the latter case, the DNA secondary structure could remain within the B family of conformations, but the change in tertiary structure might result in an altered CD spectrum. The CD spectrum of DNA in chromatin is substantially reduced in intensity relative to protein-free DNA. There is some variability in the reported extent of this alteration (7, 10-13). For isolated core particle mononucleosomes, reports agree that the CD spectrum shows a maximum ellipticity which is less than one-fourth that of protein-free DNA (8, 14, 15). The overall reductions in ellipticity measured for both chromatin and mononucleosome core particles are accompanied by subtle changes in band shape and wavelength in the spectra.

We have examined the CD properties of mononucleosomes having varying linker DNA lengths. This has allowed a deter-

mination of the relationship between the observed spectra for protein-free DNA, chromatin, and isolated mononucleosomes. We will show that CD is a useful tool with which the *extent of ordered DNA condensation* in nucleoprotein can be monitored.

METHODS

Isolation of Mononucleosomes. Mononucleosome core particles (preparation type I) were prepared from chicken erythrocyte nuclei, depleted of H1 and H5, as described (15). The nuclear digest supernatant was centrifuged at 35,000 rpm for 21 hr in a Beckman SW 40 rotor at 5°C in 5-20% linear sucrose gradients. The solvent was 10 mM Tris cacodylate/0.7 mM EDTA, pH 7.2. The pooled core particles represented approximately 25% of the material applied to the gradients. The preparations were approximately 75-80% pure; there was some contamination with subnucleosomes and mononucleosomes greater than 160 base pairs in DNA length.

Mononucleosomes with larger DNA molecular weights and varying H1 and H5 contents were prepared by an adaptation of the method of Ramsay-Shaw *et al.* (16). Chicken erythrocyte nuclei were digested at 37°C with micrococcal nuclease (EC 3.1.4.7, Worthington). The following conditions were used: 2.0-2.5 mg of DNA per ml, 45 units of nuclease per ml, a 10-min incubation (preparation type II); 2.0-2.5 mg of DNA per ml, 30 units of nuclease per ml, a 10-min incubation (preparation type III); 3.1-3.3 mg of DNA per ml, 15 units of nuclease per ml, a 5- to 10-min incubation (preparation type IV). Digestion was terminated by addition of 0.1 M EDTA (pH 7.0) to a concentration of 10 mM, and cooling on ice. The supernatant obtained after centrifugation at 12,000 × *g* for 10 min was dialyzed against 10 mM Tris-HCl/0.7 mM EDTA, pH 7.5 (Tris/EDTA) overnight. Approximately 20-50% of the nuclear DNA is found in this dialyzed supernatant, depending on the extent of digestion. The sample was concentrated by vacuum dialysis, and further dialyzed overnight against Tris/EDTA. Mononucleosomes were purified by chromatography on a Bio-Gel A-15m (100-200 mesh) column, 105 × 2.65 cm, equilibrated with Tris/EDTA, at 5°C. Pooled mononucleosome fractions were dialyzed into 0.25 mM EDTA (pH 7.0) for further study.

The yield of purified mononucleosomes depended strongly on the extent of digestion. The type II and type III preparations represented approximately 3.5-18.0% of the original nuclear DNA. The type IV mononucleosomes, however, were only 0.5-1.5% of the nuclear DNA. In part, the low yields were the result of conservative pooling of column fractions. The purity of the pooled type II, III, and IV mononucleosomes was consistently greater than 95%, as judged by electrophoretic analysis of the DNA and by rechromatography of the mononucleosomes.

Abbreviations: CD, circular dichroism; NaDodSO₄, sodium dodecyl sulfate.

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Isolation of H1- and H5-Depleted Mononucleosomes. The histones H1 and H5 were selectively removed from purified mononucleosomes by dialysis into 0.6 M NaCl/10 mM Tris-HCl/0.1 mM EDTA, pH 8.0, and chromatography on Bio-Gel A-15m in the same buffer. The salt was removed by exponential gradient dialysis to 0.25 mM EDTA (pH 7.0). No loss of other histones occurred with this procedure.

Isolation of Mononucleosome DNA. Large-scale isolation of mononucleosome DNA for spectroscopic and thermal denaturation analysis was performed essentially as described (17). Protein was digested by incubation for 1.5 hr at 37°C in the presence of 100 µg of proteinase K (Merck) per ml and 0.5% sodium dodecyl sulfate (NaDodSO₄), rather than by the usual Pronase treatment.

Gel Electrophoresis of DNA. DNA was characterized with respect to molecular weight by electrophoresis on 4% polyacrylamide slab gels, by the method of Maniatis *et al.* (18), with the Peacock and Dingman buffer system (19). Gels were stained with ethidium bromide at 0.4 µg/ml in H₂O and photographed under UV illumination. DNA molecular weight distribution and purity were also characterized by electrophoresis on 6% polyacrylamide tube gels (20). The gels were stained with 0.005% Stains-All (Eastman) in 50% formamide (pH 7.3), destained in water, and scanned at 600 nm. The scans were resolved into component contributions with a Dupont 310 curve resolver.

Gel Electrophoresis of Histones. Electrophoresis of histones was performed on Laemmli-type NaDodSO₄/13% polyacrylamide gels as described (21). Mononucleosomes were dissociated in a sample buffer containing NaDodSO₄ (22) and applied directly to the gels.

Spectroscopic Analysis. Absorption measurements were made with a Cary 14 spectrophotometer. CD spectra were recorded at 23°C with a Cary 60 and the 6001 circular dichroism accessory, as described (23).

Thermal denaturation experiments were carried out with simultaneous monitoring of the CD and absorption (dynode voltage) at 280 nm (24). In addition, for some samples the absorption changes at 260 nm, during thermal denaturation, were monitored with a Cary 14 spectrophotometer, using a jacketed quartz cell equipped with a linear thermal probe at the cell jacket exit.

RESULTS

DNA Lengths of Isolated Mononucleosomes. DNA from mononucleosome core particles (preparation type I) electrophoresed as a single band, corresponding to approximately 140 base pairs in length (Fig. 1). The type II preparation contained 32% of its DNA in the approximately 140-base-pair species, and 68% in a band averaging 170 base pairs in length. The type III preparations contained 17% of the DNA in the 140-base-pair band, and 83% in a species averaging 180 base pairs in length. Type IV mononucleosomes averaged 200 base pairs in DNA length. For the type II, III, and IV preparations, there was a real heterogeneity in DNA lengths for the species greater than 160 base pairs in length, since smaller sample loads did not appreciably reduce the observed band widths. None of the samples contained a significant amount of DNA between approximately 145 and 160 base pairs in length.

DNA isolated from the type III preparation was subjected to thermal denaturation, monitored by absorption at 260 nm, in 0.25 mM EDTA (pH 7.0). The derivative of the melting profile was a single peak, centered at 41°C, with a total hyperchromicity of 37%. This result indicates the absence of single-strand cuts in the isolated mononucleosome DNA.

Histone Composition. The histone compositions of the

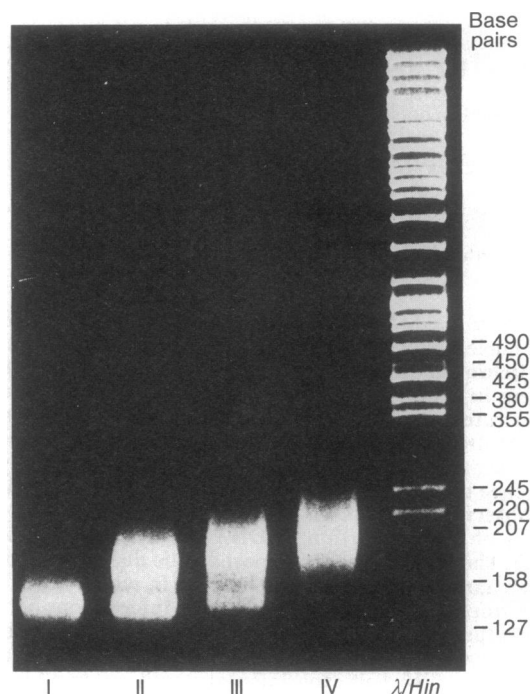


FIG. 1. A 4% polyacrylamide gel showing DNA isolated from: (I) type I mononucleosomes, (II) type II mononucleosomes, (III) type III mononucleosomes, (IV) type IV mononucleosomes. On the far right are fragments of λ DNA (Miles Laboratories), cleaved by restriction endonucleases *HincII* and *HindIII* (New England Biolabs). Sizes of these fragments are shown. The mononucleosome DNA samples were prepared by making the nucleoprotein samples 2 M in NaCl, and extracting twice with chloroform/isoamyl alcohol (24:1 vol/vol). The aqueous layer was dialyzed overnight at 5°C against glass-distilled water, lyophilized, and redissolved in one-tenth strength electrophoresis buffer.

different mononucleosome types are presented in Table 1. The core histone compositions of all mononucleosome types were identical, within experimental error, to the values obtained for chromatin or whole nuclei. The very lysine-rich histones H1 and H5 occurred at reduced levels relative to the values expected for a complete subunit of the chromatin structure. Somewhat reduced H1 and H5 contents are anticipated for preparations containing some 140-base-pair core particles. Our observed H1 and H5 contents were, however, diminished beyond the values calculated on that basis. The type II prepara-

Table 1. Histone composition of chicken erythrocyte nuclei and isolated mononucleosomes*

Nucleoprotein sample	H4	H2A	H2B	H3	H5	H1
Nuclei	1.00	0.99	1.00	1.22	0.95	0.30
Chromatin [†]	1.00	0.91	1.04	1.42	1.05	0.36
Mononucleosomes:						
Type IV (200 bp) [‡]	1.00	0.97	1.02	1.27	0.65	0.13
Type III (180 bp):						
140 bp						
= 83:17	1.00	0.96	0.98	1.23	0.61	0.17
Type II (170 bp):						
140 bp						
= 68:32	1.00	0.95	1.05	1.32	0.31	0.17
Type I (140 bp)	1.00	1.02	1.08	1.34	0	0

* Based on NaDodSO₄/polyacrylamide gels. Band areas are expressed as fractions of the area of the H4 band on the same gel. Areas were reproducible to ± 0.10 . Stained with amido black.

[†] Unpublished data.

[‡] bp, base pairs.

tion, for example, had approximately 68% of its DNA in species greater than 160 base pairs in length, but contained only 33% (0.31/0.95) of the normal H5. According to Olins and coworkers (22), chicken erythrocyte chromatin contains approximately two molecules of very lysine-rich histones for every repeat unit. Thus, even the reduced H1 and H5 levels observed here for the isolated mononucleosomes do not necessarily imply the presence of mononucleosomes greater than 160 base pairs in DNA length and devoid of H1 or H5.

Further evidence that all of the mononucleosomes that contain some linker DNA also contain some H1 and/or H5 was obtained from thermal denaturation studies. The type II, III, and IV mononucleosomes did not show any change in absorption properties (hyperchromicity) below 45°C. When, however, H1 and H5 were completely removed from the mononucleosomes, the first melting transition peak occurred at approximately 45–50°C. Since the presence of H1 or H5 is required for stabilization of the linker DNA against thermal denaturation at that temperature, this is evidence that no completely histone-free linker DNA is present.

Circular Dichroism. The CD spectra of the four mononucleosome types in 0.25 mM EDTA (pH 7.0) are shown in Fig. 2. In agreement with other reports (8, 14, 15), the 140-base-pair core particle (type I mononucleosome) has a maximum ellipticity at 284 nm, $[\theta]_{284} = 1900 \pm 200 \text{ deg}\cdot\text{cm}^2 \text{ dmol}^{-1}$. There is a shoulder at 275 nm, $[\theta]_{275} = 800 \pm 200 \text{ deg}\cdot\text{cm}^2 \text{ dmol}^{-1}$, and a negative band at 295 nm, $[\theta]_{295} = -300 \pm 200 \text{ deg}\cdot\text{cm}^2 \text{ dmol}^{-1}$. Crossover points occur at 270 nm and 292 nm. This CD spectrum is substantially altered relative to that of high mo-

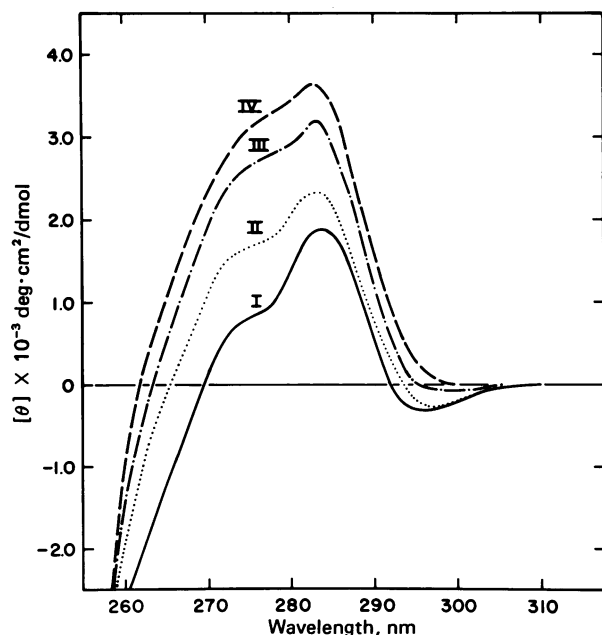


FIG. 2. CD spectra of mononucleosomes, in 0.25 mM EDTA (pH 7.0). (I) Type I mononucleosomes (140 base pairs), (II) type II mononucleosomes (170:140 base pairs = 68:32), (III) type III mononucleosomes (180:140 base pairs = 83:17), (IV) type IV mononucleosomes (200 base pairs). In all cases, the fused quartz cell path lengths were either 1 or 2 cm. Instrument sensitivity setting was 2 millidegrees for full scale. Molar ellipticities ($[\theta]$) were calculated on the basis of DNA nucleotide residue concentration, determined by a 1:1 sample dilution with 4 M NaCl, and measurement of the absorption at 258 nm ($\epsilon_{258} = 6800$). The range of concentrations used was 0.15–0.25 mM in nucleotide residues. The spectra shown are averages of three to seven spectra, obtained for at least two different preparations of each mononucleosome type.

lecular weight chromatin obtained by micrococcal nuclease digestion of chicken erythrocyte nuclei, under conditions identical to those used here for type IV mononucleosome preparation (unpublished data). The purified chromatin in the unpublished study exhibited a maximum ellipticity at 283 nm, $[\theta]_{283} = 4100 \pm 300 \text{ deg}\cdot\text{cm}^2 \text{ dmol}^{-1}$, and a strong shoulder near 275 nm, $[\theta]_{275} = 3700 \pm 300 \text{ deg}\cdot\text{cm}^2 \text{ dmol}^{-1}$.

That the main difference between the spectra of core particles and chromatin is attributable to the presence of linker DNA may be seen from the CD spectra presented here for mononucleosomes containing linker DNA. With increasing average mononucleosome DNA length (types I \rightarrow IV), the maximum ellipticity becomes greater. The observed maxima are as follows: type II, $[\theta]_{283} = 2300 \pm 300 \text{ deg}\cdot\text{cm}^2 \text{ dmol}^{-1}$; type III, $[\theta]_{283} = 3200 \pm 300 \text{ deg}\cdot\text{cm}^2 \text{ dmol}^{-1}$; and type IV, $[\theta]_{283} = 3700 \pm 300 \text{ deg}\cdot\text{cm}^2 \text{ dmol}^{-1}$. The increase in maximum ellipticity is accompanied by a change in the relative intensities of the 275 and 283 nm bands. The ratio $[\theta]_{283}/[\theta]_{275}$ decreases from 2.4 for type I mononucleosomes (core particles) to 1.2 for type IV mononucleosomes (200 base pairs average DNA length). A further trend noted in the spectra is the disappearance of the negative band near 295 nm with increasing DNA size. The mononucleosomes averaging 200 base pairs in length have a CD spectrum strikingly similar to that of high molecular weight chromatin, both in band shape and intensity.

Complete removal of H1 and H5 from chicken erythrocyte chromatin causes an increase in the ellipticity at 283 nm from 4100 to 5200 $\text{deg}\cdot\text{cm}^2 \text{ dmol}^{-1}$. The maximum ellipticities observed for the type II and IV mononucleosomes, after complete removal of H1 and H5, are approximately 4000 and 5000 $\text{deg}\cdot\text{cm}^2 \text{ dmol}^{-1}$, respectively. The near identity of the CD spectra for chromatin and type IV mononucleosomes is thus retained after removal of H1 and H5. Removal of those histones does not destroy the DNA length dependence of the mononucleosome CD spectra.

Because complete removal of H1 and H5 elevates the observed ellipticity at 283 nm, we may speculate that the reduced levels of these histones in the mononucleosome preparations may have some effect on the spectra. However, the histone composition and thermal denaturation data previously discussed indicate the presence of at least one molecule of H1 or H5 per mononucleosome greater than 160 base pairs in length. It is not known whether one molecule of H1 or H5 can have the same effect on mononucleosome DNA conformation as two molecules of these histones.

It is clear that the major determinant of the mononucleosome ellipticities is the DNA length rather than the H1 and H5 content. Thus, the 200-base-pair mononucleosomes (type IV) have a higher ellipticity than the smaller type III mononucleosomes, although the H1 and H5 contents are nearly identical. Similarly, the type III mononucleosomes have a higher ellipticity than the smaller type II species, despite a greater H1 and H5 content.

Calculated CD Difference Spectra. Fig. 3 shows the CD spectrum of protein-free DNA isolated from type III mononucleosomes, studied in the same solvent used for mononucleosomes (0.25 mM EDTA, pH 7.0). This spectrum is in agreement with spectra we obtained for high molecular weight chicken erythrocyte DNA, and with a published spectrum of chicken erythrocyte core particle DNA (15), in the same solvent. The maximum ellipticity occurs at 275 nm ($[\theta]_{275} = 9300 \pm 200 \text{ deg}\cdot\text{cm}^2 \text{ dmol}^{-1}$). There is a strong shoulder at 280 nm ($[\theta]_{280} = 9000 \pm 200 \text{ deg}\cdot\text{cm}^2 \text{ dmol}^{-1}$).

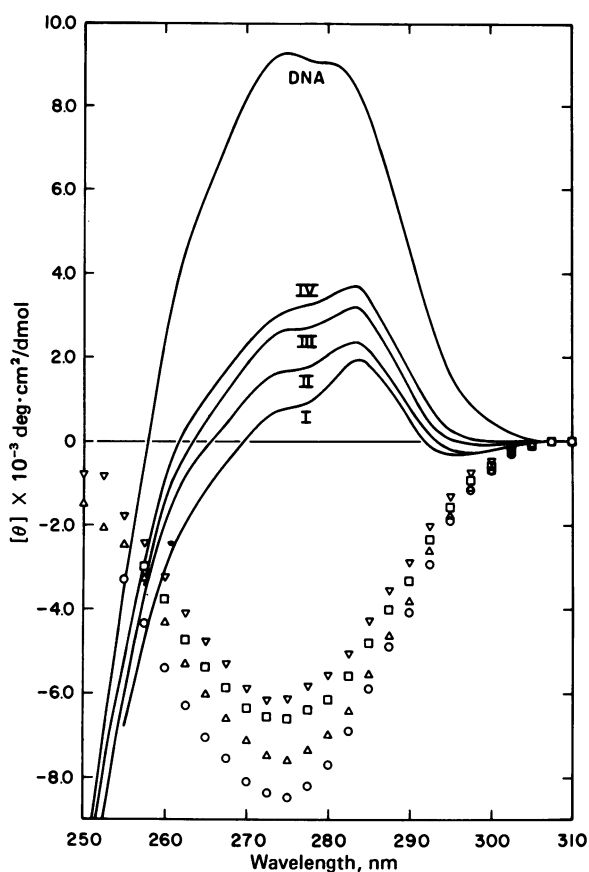


FIG. 3. CD spectra of DNA and mononucleosomes, and calculated difference spectra. Solvent was 0.25 mM EDTA (pH 7.0). Ellipticity calculated on the basis of DNA nucleotide residue concentration. Curves I, II, III, and IV correspond to the four mononucleosome types, increasing in average DNA length. Difference spectra points were calculated by subtracting the spectrum of purified mononucleosome DNA, obtained under identical conditions, from each of the mononucleosome spectra. O, Type I minus DNA; Δ , type II minus DNA; \square , type III minus DNA; ∇ , type IV minus DNA.

The CD spectra of the mononucleosomes have been redrawn on Fig. 3 to emphasize the relationship between each of these spectra and that of the protein-free DNA. A smaller positive ellipticity is invariably accompanied by a larger $[\theta]_{284}/[\theta]_{275}$ ratio and a larger negative band at 295 nm. The reason for these effects is shown by calculating difference spectra between each of the mononucleosome spectra and that of protein-free DNA. The data points shown in Fig. 3 were obtained by simple subtraction. In each case, the difference spectrum is a single negative band centered near 275 nm. Each of the mononucleosome spectra may thus be regarded as the net result of the normal CD contribution observed for B-form DNA and the additional negative ellipticity contribution from this 275 nm band. The position of the negative band is such that its presence not only reduces the apparent positive CD maximum, but also shifts it from 275 nm for protein-free DNA to 284 nm for core particles. It is also responsible for the appearance of the negative band at 295 nm.

The intensity of the difference band is related to the mononucleosome DNA size, being greatest for the core particles. The negative band contribution for the 200-base-pair mononucleosomes is approximately 73% of that for 140-base-pair core particles. This value may be related to the proportion (140/200 = 70%) of DNA involved in the core structure.

DISCUSSION

Correlation of Nucleoprotein CD Properties with DNA Condensation. In this study, we have found that the CD spectra of isolated mononucleosomes, in low ionic strength solution, are strongly dependent on the DNA length. Core particles exhibit a low $[\theta]_{284}$ value. Mononucleosomes which more closely approximate the size of an average chromatin subunit exhibit a spectrum strikingly similar to that observed for chromatin isolated and studied under identical conditions. In all cases studied, the mononucleosome CD spectra were shown to be resolvable into a contribution identical to that of free DNA in solution and a negative band centered at 275 nm. Shih and Lake (11) had previously observed that the CD spectrum of chromatin is also resolvable into these components. To a first approximation, the intensity of the added negative band is correlated with the fraction of the average DNA length that is directly involved in the nucleosome core. The calculated magnitude of the negative band is also dependent on the presence of the very lysine-rich histones H1 and H5. Removal of these proteins reduces the negative contribution somewhat and thus increases the observed positive ellipticity.

From these data, we may conclude that, under our experimental conditions of very low ionic strength, only that fraction of the DNA that is directly histone-bound contributes to the change in CD properties. The remaining linker DNA has the normal B DNA spectrum. This is true despite the apparent stabilization of all linker DNA to thermal denaturation. Since the extent of linker DNA condensation depends on ionic strength, the presence of H1, and the method of nucleoprotein isolation (4–7, 12, 13, 25–27), we consider it likely that most of the linker DNA studied herein is not condensed with the core DNA. Clearly, it is not involved in the particular DNA arrangement that gives rise to the negative CD band at 275 nm. We propose that the nucleosome core DNA, condensed into a unique ordered arrangement, exhibits the negative CD band contribution. The fraction of linker DNA so condensed by H1 and H5 also contributes in a similar fashion. DNA not so condensed has the same spectral properties as free DNA. Consideration of available data in the literature shows the correlation between DNA condensation and CD alteration to be general.

From electron microscopic and hydrodynamic studies, it has been concluded that chromatin becomes more compact as the ionic strength of the solvent is increased up to certain levels. Parallel studies correlating the extent of this condensation with CD spectra have shown that the positive ellipticity above 250 nm is reduced as the chromatin compacts (26, 27). It is also possible to isolate chromatin, under very mild conditions, that is more highly condensed than chromatin isolated by conventional methods (7, 12, 13). The condensed chromatin samples exhibit low-magnitude CD spectra. These CD spectra are essentially identical to our spectra for mononucleosomes with very little linker DNA. We interpret these results to mean that appropriate condensation of linker DNA with the core causes its spectral contribution to become identical with that of the core DNA.

Recently, several reports (8, 28, 29) have described the isolation of oligonucleosomes with little or no linker DNA between cores. These "tight oligomers" exhibit low-magnitude CD spectra virtually identical to the spectrum for core particle mononucleosomes. Again, this observation may be readily explained by noting that in such species all of the DNA should be condensed and thus have the same intensity for the negative CD band at 275 nm. Oligonucleosomes with linker DNA between cores will invariably exhibit greater positive ellipticities unless the solvent system or the particular isolation procedures used result in linker DNA condensation.

Secondary and Tertiary Structure Contributions to CD of DNA. The alteration in CD properties that accompanies DNA condensation by histones could be due to a change in secondary structure or to some aspect of the newly formed tertiary structure. Raman spectroscopic (30) and wide-angle x-ray scattering studies (31) have shown that DNA in chromatin and isolated nucleosomes adopts a secondary structure within the B family of conformations. Protein-free DNA in low ionic strength aqueous solution also exists in a B secondary structure (31, 32). The extent of any difference between these B conformations has not been firmly established. It has been proposed (33) that DNA within the nucleosome core has exactly 10.0 base pairs per helical turn, and that free DNA in solution has closer to 10.7 base pairs per turn. Theoretically, a conformational change of this magnitude should not result in a large alteration in CD properties of DNA unless there are substantial changes in base tilt with respect to the helix axis (34). B DNA in films, prepared under conditions shown by x-ray studies to yield a structure with 10 base pairs per turn, exhibits a CD spectrum essentially identical to that of DNA in solution (35).

An alternative explanation for the CD properties of nucleosomal DNA can be made by consideration of the unique tertiary structure. A single negative band at 275 nm, such as that found here in the calculated difference spectra, has previously been observed for DNA under several conditions. Examples are Ψ DNA (36), DNA-H1 complexes (23), and DNA-poly(L-lysine) complexes (37). In each of these cases, the secondary structure of the DNA remains of the B type (32, 37), but the DNA is condensed into a compact, possibly microcrystalline, arrangement. The specific ordering of the condensed DNA is required for the observation of the unique CD spectra, since destruction of that order by mild heating, under conditions that do not disrupt the aggregates themselves, results in the typical CD spectrum for B DNA (38). The 275 nm band is apparently the result of the interaction of circularly polarized light with the ordered tertiary arrangement.

Although it has been proposed (36) that interactions among many adjacent strands of DNA in large particles are required for the Ψ CD spectrum, it may be possible that the interaction between two DNA strands on the nucleosome is sufficient to produce a low-intensity effect. According to the model for nucleosome structure determined by Finch and coworkers (33), the edge-to-edge distance between adjacent turns of the DNA superhelix is approximately 6 Å. We propose that the CD spectra of nucleosomes contains a Ψ -like contribution (the 275 nm negative band), which arises as a consequence of the interaction of circularly polarized light with the highly ordered DNA tertiary structure and which depends on the proximity of the adjacent superhelical turns. A consequence of this interpretation is that separated (noncondensed) supercoils of DNA will show no Ψ -type CD contribution. This is in agreement with the CD data for supercoiled closed circular DNA (39).

Therefore, the interpretation is favored herein that the tertiary arrangement of nucleosomal DNA contributes to the observed CD spectrum. On the basis of our data, however, we cannot completely discount a contribution from small secondary structure alterations, which have not been detected by Raman (30) and wide-angle x-ray (31) studies.

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