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The Conformation of Proteins in Chromatin. A Circular Dichroism Study below 250 nm†

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ABSTRACT: This paper is an investigation of the circular dichroism (CD) spectra of DNA and protein in chromatin. The circular dichroism (CD) of chromatin below 250 nm is due to DNA and protein peptide chromophores. The spectrum in this region is resolved into contributions from salt-extractable proteins (histone and non-histone proteins extractable with sodium chloride), residual non-histone proteins (not extractable with 3 *M* sodium chloride), and DNA. Below 250 nm, DNA in chromatin has the same CD spectrum as DNA free in solution, in contrast to the CD of DNA above 250 nm (Hjelm, R. and Huang, R. C., (1974), *Biochemistry 13,* 5275). Histones and salt-extractable non-histone proteins in chromatin are seen to have an average CD like those observed for globular proteins. The average CD of the residual non-histone proteins is consistent with a population of proteins with more extended conformation. The CD of each of these components is found to be the same in chromatins isolated from tissues having different nuclear synthetic activities: chick embryo brain, pig cerebellum, myeloma K41, calf thymus, and chicken erythrocyte.

Isolated interphase chromosomes of higher plants and animals, chromatin, are a complex of DNA, proteins, and some RNA (Stellwagen and Cole, 1969; Hearst and Botchan, 1970; DeLange and Smith, 1971; Elgin et al., 1971; Huang and Hjelm, 1975). Among the chromatin proteins two classes are readily distinguished: the small, basic, well-defined histones and the heterogeneous non-histone proteins. Although much study has been devoted to the problem of chromatin structure and to the conformation of DNA in chromatin, little attention has been paid to the structure of protein in chromatin. To obtain some information on the structure of proteins in chromatin, in this paper, we extend our earlier studies on the circular dichroism of chromatin above 250 nm (Hjelm and Huang, 1974, 1975) into the region of the spectra between 200 and 250 nm.

From the studies on the CD of chromatin above 250 nm—where the signal is mainly due to DNA—we learned that the interaction of the histone proteins with DNA results in secondary structure of DNA in chromatin that is different from the conformation of DNA in solution at very low ionic strength (Shih and Fasman, 1970; Permogarov et al., 1970; Henson and Walker, 1970). Observations of the CD spectrum of chromatin between 200 and 250 nm—where protein peptide and DNA chromophores contribute—have indicated that the proteins in chromatin may contain considerable secondary structure not found in the isolated material at low ionic strength (Permogarov et al., 1970; Henson and Walker, 1970; Fric and Sponar, 1971; Ramm et al., 1972). No effort, however, was made to resolve the signal in this region into contributions from different chromatin components. Their conclusion on the secondary structure of proteins in chromatin was, therefore, based on indirect evidence.

We have attempted to separate the CD contribution of chromatin DNA from that of chromatin proteins. In this paper, we report the resolution of the CD spectra of chromatin between 200 and 250 nm into contribution from three components: salt soluble proteins (histones and salt-extractable non-histones), residual non-histone proteins, and DNA. In chromatin the salt-extractable non-histone and histone proteins have very similar CD spectra and are like those observed for globular proteins. The CD of the residual non-histone proteins is unlike that of the salt-soluble proteins and is consistent with a structure that is more extended than the other chromosomal proteins. The contribution of the DNA to the CD in this region is indistinguishable from the spectrum of free DNA in solution. The spectra for the three protein classes and DNA appear to be the same in all the chromatins studied, regardless of composition of origin.

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Experimental Section

Materials

Calf thymus (Henry W. Stapf, Inc.) and pig cerebellum (Esskay Quality Meat Company) are obtained immediately after slaughter and placed on ice. Blood from 6-month-old hens is collected from a neck wound and placed in 10% (w/v) trisodium citrate solution (100 ml of solution/1000 ml of blood) after the method of Murray et al. (1968). Myeloma tumors are cultured in vivo in BALB/c mice, and are provided by the courtesy of Mrs. Susan Auyang and Dr. Janet Stavnezer. Chick embryo brains are dissected from 11-day-old embryos. All materials are used immediately, with the exception of calf thymus which is often stored at −20° until use.

Methods

Isolation of Nuclei. Nuclei from chick embryo brain, pig cerebellum, myeloma K41, and chicken erythrocytes are obtained as previously described (Hjelm and Huang, 1974, 1975).

Isolation of Chromatin. Chromatin is isolated by the method of Huang and Huang (1969). The starting material is isolated nuclei except for calf thymus where finely chopped tissue is used.

Isolation and all succeeding operations are carried out at 4°, unless otherwise noted.

Low and High Salt Dissociation of Chromatin. Chromatins are dissociated with NaCl–5 m*M* phosphate of ionic strengths 0.2–0.35, 0.6, 1.0, 1.2, and 3.0 as described elsewhere (Hjelm and Huang, 1974, 1975). The nomenclature for the dissociated nucleoproteins is the same as used previously (Hjelm and Huang, 1974, 1975): nucleoprotein low salt refers to chromatin dissociated with NaCl–5 m*M* phosphate solutions of low ionic strength (0.2–0.35); nucleoprotein 0.6, nucleoprotein 1.0, nucleoprotein 1.2, and nucleoprotein 3.0 refer to nucleoproteins produced by dissociation of chromatins with NaCl–5 m*M* phosphate solutions of ionic strengths 0.6, 1.0, 1.2, and 3.0, respectively.

Quantitative Determination of Chromatin Components. Protein determinations are made by the method of Lowry et al. (1951). The DNA content of the samples is estimated by the diphenylamine procedure of Burton (1956). Lowry determinations are made directly on the nucleoprotein solutions, and the diphenylamine procedure is carried out on the hot 5% perchloric acid soluble fraction (70°, 15 min).

Measurement of Circular Dichroism. Sheared samples are exhaustively dialyzed against 0.7 m*M* phosphate (pH 6.8). CD spectra are measured on a Cary 60 spectropolarimeter with a 6003 CD attachment. Cells have a 10-mm path length. All sample spectra are measured at 4°.

Calculations. CD data are collected at 2.5-nm intervals from the chart readout of the Cary 60. The measured ellipticity, θ^{λ}, at each wavelength, λ, is reduced to molar ellipticity, $[\theta]^{\lambda}$, by the equation

$$[\theta]^{\lambda}_{i} = (M_i/c_i l 10)\theta^{\lambda}$$

where c_i is the concentration of i in grams per cm^3, l is the cell path length, and M_i is the residue molecular weight of i. M_i is taken to be 115 for protein and 309 for DNA. Units of molar ellipticity, $[\theta]^{\lambda}_{i}$, are deg cm^2/dmol of i.

Data reduction is carried out on a Hewlett-Packard 2100 computer.

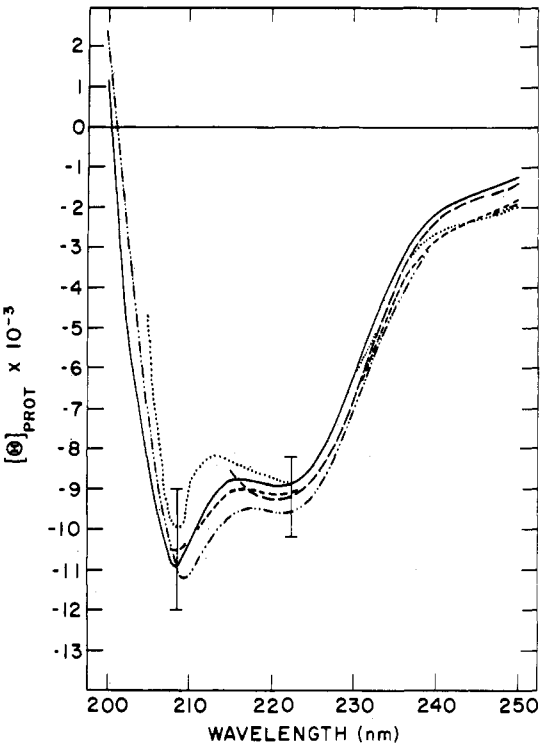

FIGURE 1: Circular dichroism below 250 nm of chromatins. (–) Chick embryo brain; (– –) pig cerebellum; (– · · –) calf thymus; (– · – ·) myeloma; (· · ·) chicken erythrocyte. Molar ellipticities are expressed as deg cm^2/dmol of protein. Error bars indicate ± 1 SD. In these CD spectra, standard deviation is about 10% of the signal over the entire spectral range.

Results

Circular Dichroism below 250 nm of Chromatins. The chromatins studied are isolated from tissues demonstrating different capacities for RNA and DNA synthesis: calf thymus, myeloma, and chick embryo brain synthesize both RNA and DNA; pig cerebellum produces only RNA—like other adult neural tissues (Deluca et al., 1953; Bendick et al., 1953; Leblond and Walker, 1956; Koenig, 1958); and chicken erythrocyte is an example of a cell type which is completely repressed in nuclear synthetic activity (Williams, 1971; Neelin et al., 1964). The chromatins also differ in the relative content of RNA and non-histone proteins (Hjelm and Huang, 1975). Regardless, the spectra below 250 nm are quite similar when the molar ellipticities are expressed as deg cm^2/dmol of protein (Figure 1). Below 235 nm the spectra are indistinguishable and include two negative extrema: one at 208 nm of about −10,500 deg cm^2/dmol of protein; another at 223 nm of approximately −10,500 deg cm^2/dmol of protein. These values are close to those reported for calf liver chromatin (Simpson and Sober, 1970). Above 235 nm small, but significant, differences are apparent (Figure 1): pig cerebellum and chick embryo brain chromatin spectra are less negative in this region than spectra of the other three chromatins; this is probably due to the relatively large contribution of DNA to the CD in this region (vide infra).

It should be noted that in highly concentrated samples of pig cerebellum and chick embryo brain chromatins the minimum at 208 nm is not observed, there apparently being a concentration effect. However, less concentrated solutions of pig cerebellum and chick embryo brain chromatins, all solutions of chromatin from the other tissues studied, are

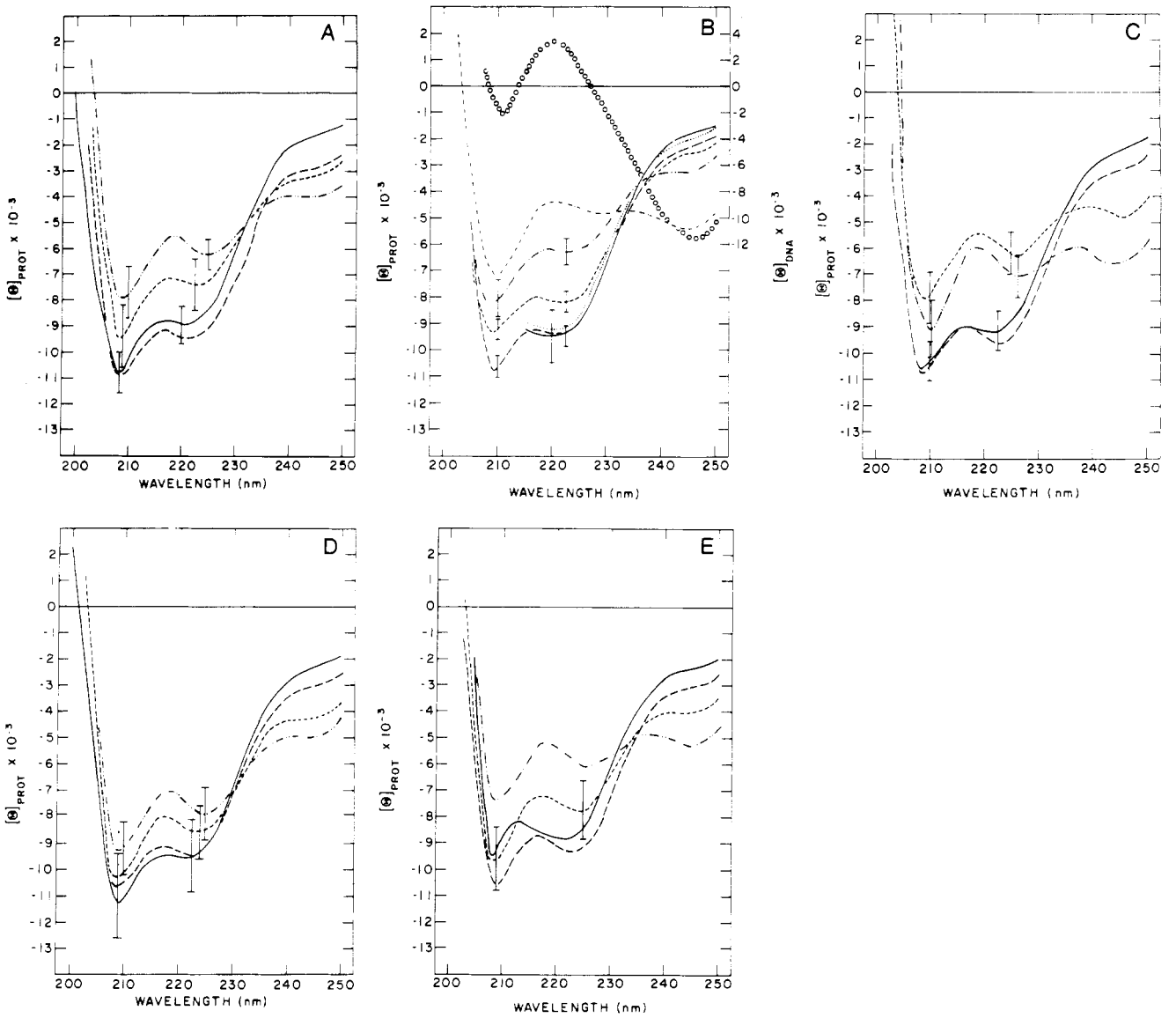

FIGURE 2: (A) Circular dichroism below 250 nm of chick embryo brain and nucleoproteins. (–) Chromatin; (– –) nucleoprotein 0.6; (– - –) nucleoprotein 1.0; (– • – –) nucleoprotein 1.2. Molar ellipticities are expressed as deg cm^2/dmol of protein. Error bars represent ± 1 SD. (B) Circular dichroism below 250 nm of pig cerebellum chromatin and nucleoproteins. (–) Chromatin; (• • •) nucleoprotein low salt; (– –) nucleoprotein 0.6; (– - –) nucleoprotein 1.0; (– • – –) nucleoprotein 1.2; (– - –) nucleoprotein 3.0; (O) DNA. Molar ellipticities of chromatin and depleted nucleoprotein fractions are expressed as deg cm^2/dmol of protein. Molar ellipticities of the DNA are expressed as deg cm^2/dmol of DNA. DNA is in 0.7 m*M* phosphate (pH 6.8); note difference in scales. (C) Circular dichroism below 250 nm of myeloma K41 chromatin and nucleoproteins. (–) Chromatin; (– –) nucleoprotein 0.6; (– - –) nucleoprotein 1.0; (– • – –) nucleoprotein 1.2. (D) Circular dichroism below 250 nm of calf thymus chromatins and nucleoproteins. (–) Chromatin; (– –) nucleoprotein 0.6; (– - –) nucleoprotein 1.0; (– • – –) nucleoprotein 1.2. (E) Circular dichroism below 250 nm of chicken erythrocyte chromatin and nucleoproteins. (–) Chromatin; (– –) nucleoprotein 0.6; (– - –) nucleoprotein 1.0; (– • – –) nucleoprotein 1.2.

far less turbid than the highly concentrated pig and chick brain samples suggesting that effects due to turbidity (Gordon, 1972; Holzwarth et al., 1974) may contribute to the absence of the 208 extremum in the concentrated pig and chick chromatin samples.

It appears then that the CD spectra below 235 nm are insensitive to the origin, and composition of the chromatins, in contrast to the spectra in the region above 260 nm (Hjelm and Huang, 1974, 1975).

Circular Dichroism below 250 nm of Chromatins Dissociated with NaCl–5 mM Phosphate Solutions. In our previous studies (Hjelm and Huang, 1974, 1975) we have determined the amount and type of proteins removed at different ionic strengths from the various chromatins studied here. It was found that each chromatin responds differently to NaCl dissociation, but a general pattern was discerned; low salt (0.35 ionic strength) removes approximately 50% of the non-histone proteins. These and the lysine rich histone (I) are removed with NaCl–5 m*M* phosphate solution of 0.6 ionic strength. Using 1.0 and 1.2 *M* NaCl, 50% of the non-histones, histone I, and variable amounts of the slightly lysine rich histones (IIb_1, IIb_2) and arginine rich histone (III) are removed. All histones including histone IV and all the salt-extractable non-histone proteins are removed with 3.0 *M* NaCl. The proteins not removed under these conditions are the residual nonhistone proteins.

The CD spectra of chromatins and chromatins dissociated with NaCl–5 m*M* phosphate solutions of different ionic strengths are shown in Figure 2. Removal of lysine-rich histone and a large fraction of the non-histone proteins with 0.6 *M* NaCl (Hjelm and Huang, 1974) results in no significant changes in the molar ellipticities (expressed as deg

cm^2/dmol of protein) below 235 nm in any of the samples (Figure 2). This result is in contrast to those of Ramm et al. (1972) and Fric and Sponar (1971) who observed a significant change in the CD of this region with 0.7 *M* NaCl dissociation of calf thymus chromatin. No change in the spectrum is seen in pig cerebellum nucleoprotein low salt (dissociated with 0.2–0.35 *M* NaCl–5 m*M* phosphate) samples (Figure 2). Fifty percent of the non-histone proteins are removed in this case (Hjelm and Huang, 1975).

When dissociation is carried out with NaCl–5 m*M* phosphate solutions of ionic strength 1.0 and 1.2, the CD in the region below 235 is seen to become less negative (Figure 2). Furthermore, differences among the nucleoprotein 1.0 and nucleoprotein 1.2 samples from different chromatins are observed throughout the entire far 250-nm spectra (Figure 2).

The spectrum of each of nucleoprotein 3.0 samples from the various chromatins is different. The spectrum of pig cerebellum nucleoprotein 3.0 is given as an example (Figure 2B). It is representative in that the CD of all the nucleoprotein 3.0 are different from the CD of DNA (F).

Regardless of the apparent differences observed in the CD of the variously depleted nucleoproteins, some general characteristics apply to all the spectra (Figure 2). The two negative extrema observed in chromatins (Figure 1) are maintained in the nucleoprotein low salt 0.6, 1.0, and 1.2 samples (Figure 2). The 223-nm minimum of chromatin is seen to red-shift to 228 nm in the samples dissociated with greater than 0.6 *M* salt. All spectra show a depression of the spectrum in the region above 235 nm. These effects are due to the increase in the contribution of DNA to the spectrum as the protein/DNA ratio decreases (vide infra).

Analysis of Spectra. If the CD spectrum of each sample is normalized to its DNA content, (rather than its protein content as in Figures 1 and 2) meaningful and surprisingly simple results are obtained. Here, we chose (as a matter of convenience) to use the ellipticity of each sample at 250 nm, θ^{250} as the indicator of DNA content; thus in the analysis given below the spectrum of each sample is divided by its ellipticity at 250 nm ($\theta^{\lambda}/\theta^{250}$).

We rationalize this choice of normalization by the fact that the ellipticity at 250 nm has no contribution from the histone proteins (Fasman et al., 1970) and that the non-histone side chain and RNA chromophores contribute no more than 5% of the signal (Hjelm and Huang, 1975); thus the ellipticity at this point is almost entirely due to DNA. More important, our experience is that this region of the DNA spectrum is insensitive to the conformation changes which occur in DNA as proteins are removed from the chromatins (Hjelm and Huang, 1974). Also, this method of normalization has the advantage of making the independent measurements determining the ordinate and abscissa of each point in Figures 3 and 5. When DNA content as measured by diphenylamine (Burton, 1956) is used to normalize the data, the same results as outlined below are obtained. The spectral ratio $\theta^{\lambda}/\theta^{250}$, for all the chromatins and depleted nucleoproteins vs. their protein/DNA molar ratio at wavelengths 210, 220, and 230 nm, is plotted in Figure 3. The plots are highly suggestive of a linear relationship between the $\theta^{\lambda}/\theta^{250}$ spectral ratio and the protein/DNA molar ratio. This is expected, in part, in that the molar ellipticities of the chromatin, nucleoprotein low salt, and nucleoprotein 0.6 samples, when expressed as deg cm^2/dmol of protein (Figures 1 and 2), show no variation among themselves below 235 nm. These samples contribute the points at molar ratios

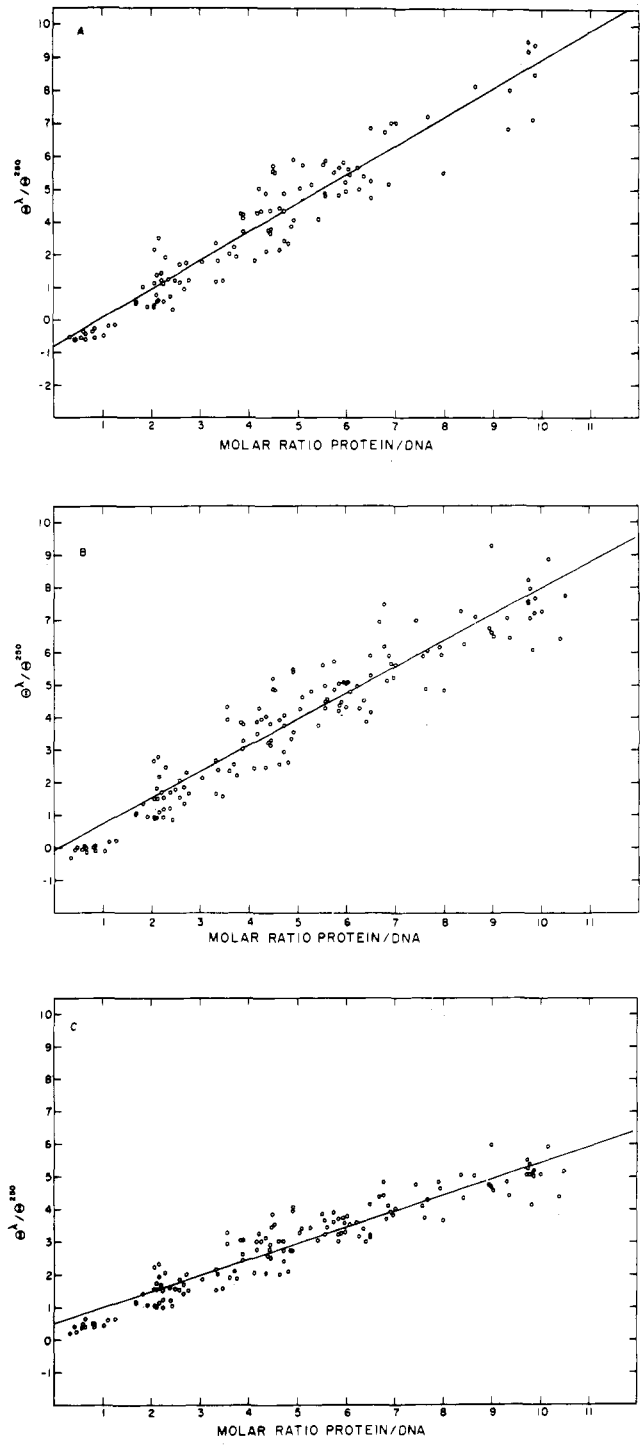

FIGURE 3: Spectral ratios, $\theta^{\lambda}/\theta^{250}$, of chromatins and nucleoproteins vs. protein/DNA molar ratio. Least-squares fit of data: (A) 210 nm; slope, 0.87 ± 0.03; intercept, −0.17 ± 0.14; (B) 220 nm; slope, 0.80 ± 0.03; intercept, −0.09 ± 0.14; (C) 230 nm; slope, 0.49 ± 0.02; intercept, 0.48 ± 0.09.

above 5.0 (Figure 3). It is apparent, also, from Figure 3 that the spectral data for the nucleoprotein 1.0 and nucleoprotein 1.2 samples, occurring between protein/DNA mass ratios of 2 and 5, do not differ significantly from the linearity established by the chromatin, nucleoprotein low salt, and nucleoprotein 0.6 samples; regardless of the fact that the spectra of nucleoprotein 1.0 and nucleoprotein 1.2 when expressed as deg cm^2/dmol of protein show considerable variation among the various chromatin sources (Figure 2). Plots for all wavelengths measured between 200 and 250 nm are

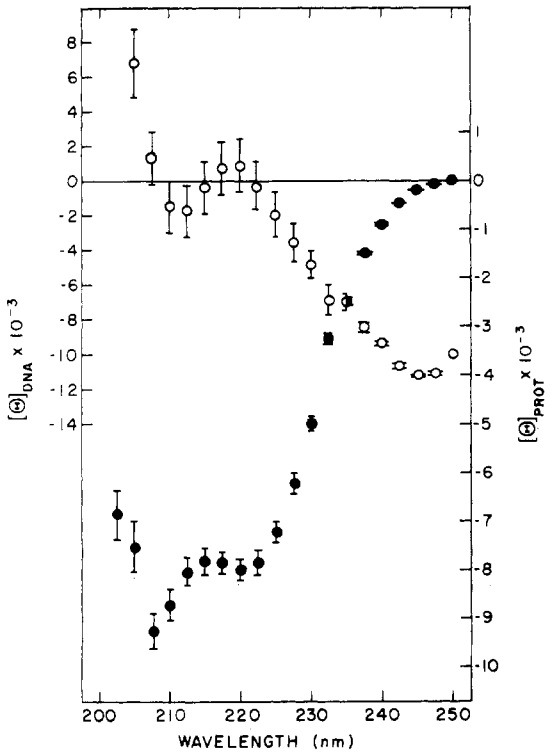

FIGURE 4: Circular dichroism below 250 nm of DNA and salt-extractable proteins. (○) DNA; (●) protein. A regression analysis is made of the $\theta^{\lambda}/\theta^{250}$ vs. protein/DNA mole ratio at each wavelength (see Figure 3). Values of the intercept and slope multiplied by $[\theta]^{250} = -10{,}030$ give the ellipticities for DNA and protein, respectively. Errors are given from the respective square root variance of the regression analysis, multiplied by $-10{,}030$.

linear even in the region above 235 nm where all samples are dissimilar. The lines shown on Figure 3 are determined by least-squares fit of the data.

The simplest model which will explain the apparent linear behavior of $\theta^{\lambda}/\theta^{250}$ is

$$\theta^{\lambda}/\theta^{250} = (C_{Prot}/C_{DNA})[\theta]^{\lambda}_{Prot}/[\theta]^{250}_{DNA} + [\theta]^{\lambda}_{DNA}/[\theta]^{250}_{DNA} \quad (1)$$

An implication of eq 1 is that the ellipticity of any given chromatin or partially depleted nucleoprotein sample can be represented by the linear form $\theta^{\lambda} = C_{Prot}[\theta]^{\lambda}_{Prot} + C_{DNA}[\theta]^{\lambda}_{DNA}$, where θ^{λ} is the measured ellipticity at wavelength, λ; C_{Prot} and $[\theta]^{\lambda}_{Prot}$ are the concentration (in dmol/cm^3) and the molar residue ellipticity at λ (deg cm^2/dmol) of the protein, respectively; C_{DNA} and $[\theta]^{\lambda}_{DNA}$ are the corresponding quantities for DNA. Thus, the molar ellipticities (deg cm^2/dmol of protein) shown in Figures 1 and 2 can be represented by the equation

$$\theta^{\lambda}/C_{Prot} = [\theta]^{\lambda}_{Prot} + (C_{DNA}/C_{Prot})[\theta]^{\lambda}_{DNA}$$

where the ratios $[\theta]^{\lambda}_{DNA}/[\theta]^{250}_{DNA}$ and $[\theta]^{\lambda}_{Prot}/[\theta]^{250}_{DNA}$ both are independent of the protein/DNA ratio, implying that neither the CD of DNA nor chromosomal proteins change as protein is removed from chromatin. The least-squares fit of eq 1 to the points in Figure 3 are indicated in the legend to that figure.

Equation 1 indicates that the slopes of the plots in Figure 3 at different wavelengths will give an average CD spectra for the salt-soluble chromosomal proteins, and the intercepts will give the spectrum of the DNA. These are illustrated in Figure 4. The derived CD of DNA below 250 nm (Figure 4) is very close to that observed experimentally for DNA free in solution at low ionic strength (Figure 2B). The protein CD is similar to those obtained from globular proteins. However, this equation cannot explain all the observations (Figures 1 and 2): the equation becomes more negative in the regions where the DNA ellipticity is negative as the protein to DNA ratio is decreased. This is observed in Figure 2 above 235 nm, but below this point there are regions where the DNA is supposedly negative (Figures 2B and 4), where the CD is seen to increase as the protein/DNA ratio increases. Examination of Figure 3 shows that over most of the molar ratio scale the experimental points fit the least-squares lines well. Below values of 2.0, however, all the points are seen to lie below the line. This is true of plots of all the wavelengths sampled. The points which lie below the line correspond to samples dissociated with 3.0 ionic strength NaCl–5 m*M* phosphate (nucleoprotein 3.0), and thus contain only residual non-histone proteins. A plot of the $\theta^{\lambda}/\theta^{250}$ ratio vs. the protein/DNA molar ratio of the nucleoprotein 3.0 samples (Figure 5) is consistent with a linear relationship between the two parameters, and an equation analogous to eq 1 describes the data. Thus, it is reasonable to assume that the residual non-histone proteins from the various chromatins have the same average conformational properties. The derived DNA spectrum (Figure 6) of the least-squares lines is indistinguishable from experimentally observed spectra (Figure 2B). The slopes of the least-squares lines are shallower (Figure 5) than those derived for the salt-soluble proteins (Figures 3 and 4); thus the resultant CD is of lesser magnitude (Figure 6). We shall see that this spectrum is reasonable for protein of somewhat extended conformation.

Our model for the CD data of chromatins and depleted nucleoproteins is now

$$\theta^{\lambda} = C_s[\theta]^{\lambda}_s + C_r[\theta]^{\lambda}_r + C_{DNA}[\theta]^{\lambda}_{DNA} \quad (2)$$

where the subscripts s and r refer to the soluble and residual proteins, respectively. As in eq 1, all the ellipticities, $[\theta]\lambda$, are assumed to be constant. The form of eq 2 indicates that the intercept of the linear regression lines of the salt-extractable proteins (Figure 3) need not necessarily give a CD spectrum for DNA. That the spectrum obtained for DNA in Figure 4 is reasonable is due to the salt-soluble proteins being the dominant source of the CD of chromatin below 250 nm; thus the unresolved CD in this region is essentially characteristic of these chromosomal proteins.

If the three-term linear model of the CD of chromatin below 250 nm, eq 2, is an accurate description of the data, then by using the model it should be possible to predict the content of salt-extractable and residual proteins in chromatins and nucleoproteins. Equation 2 becomes

$$[\theta]^{\lambda}_{Prot} = \theta^{\lambda}/C_T = f_r[\theta]^{\lambda}_r + f_s[\theta]^{\lambda}_s + (C_{DNA}/C_T)[\theta]^{\lambda}_{DNA} \quad (3)$$

when divided by the total protein concentration of the sample C_T. f_r and f_s are the fractions of the total protein that are residual and salt-soluble chromosomal proteins, respectively. A least-squares fit to eq 3 of the various nucleoprotein and chromatin CD spectra (Figures 1 and 2) using the ellipticities calculated for the salt-soluble proteins (Figure 4), residual non-histone proteins (Figure 6), and DNA (from the intercept of the regression line of the residual non-histone proteins, Figure 6), with the condition that $f_r + f_s = 1$, should yield values for protein/DNA mass ratios close to those measured (Table I). Furthermore, the solu-

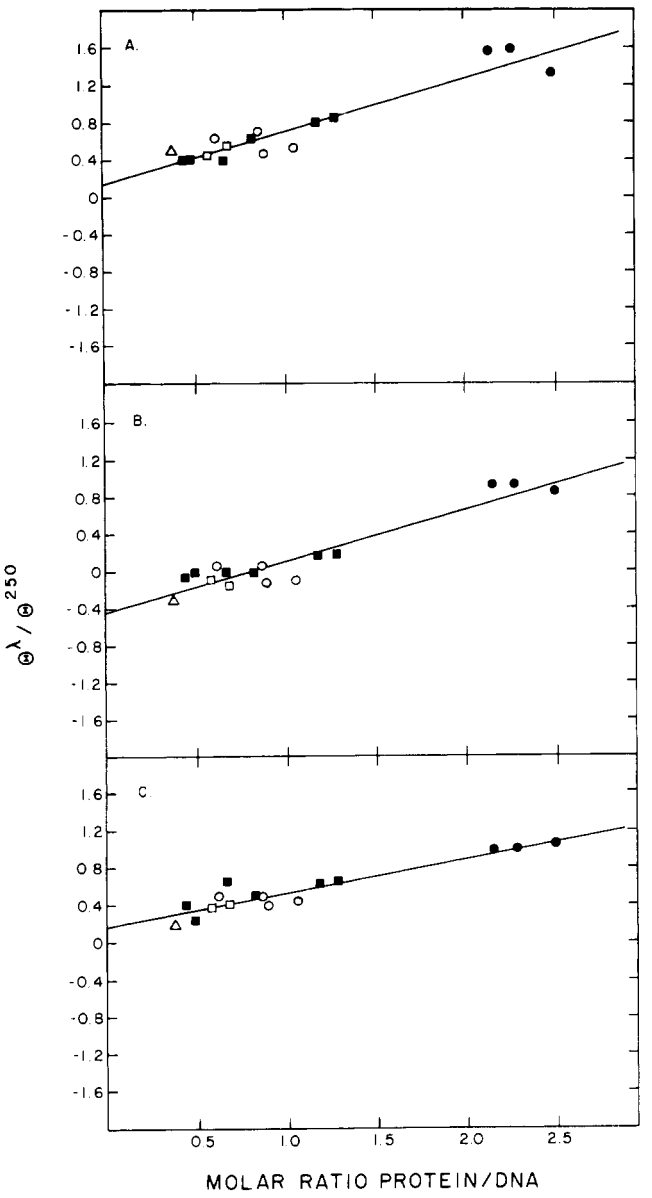

FIGURE 5: Spectral ratios, $\theta^{\lambda}/\theta^{250}$, of nucleoprotein 3.0 samples vs. protein/DNA molar ratios. Least-squares analysis of data: (○) chick embryo brain; (●) pig cerebellum; (□) myeloma K41; (■) calf thymus; (△) chicken erythrocyte. (A) 210 nm; slope, 0.56 ± 0.05; intercept, 0.10 ± 0.06; (B) 220 nm; slope, 0.56 ± 0.05; intercept, −0.43 ± 0.06; (C) 230 nm; slope, 0.36 ± 0.04; intercept, 0.19 ± 0.05.

tions should give reasonable estimates of the salt-soluble protein and residual non-histone protein/DNA mass ratios. The fit is made over the interval 205–250 nm.

The results (Table I) give total protein/DNA mass ratios which are in good accord with the experimentally determined values. Also, the amount of residual non-histone proteins calculated for nucleoprotein 3.0 samples agrees with the experimentally determined result (Table I). It is apparent, however, that the amount of residual non-histone protein detected in some other samples is only in fair agreement with the amount found to be present in the corresponding nucleoprotein 3.0 samples. This is especially true in the samples having relatively large protein/DNA mass ratios where none of this component is detectable. To some extent this is not surprising in that the residual non-histone proteins will not be revealed unless they contribute at least 10% of the total sample signal. In cases where the errors are

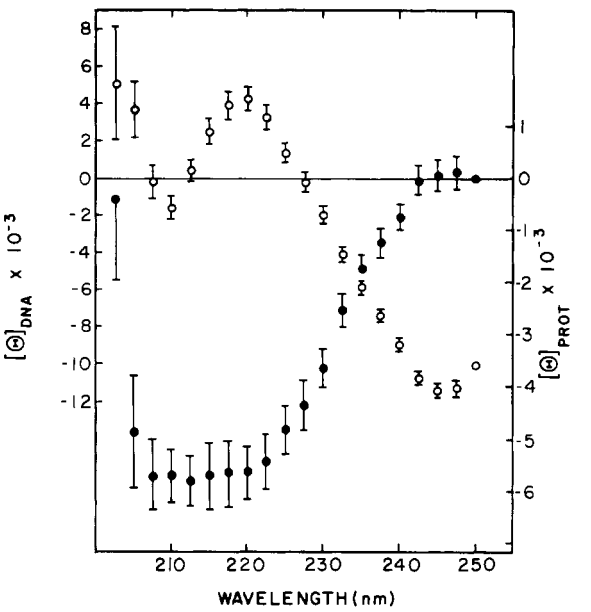

FIGURE 6: Circular dichroism below 250 nm of DNA and residual non-histone proteins. (○) DNA; (●) residual non-histone proteins. Values and errors calculated from least squares like those in Figure 5.

very large the non-histone protein may have to contribute considerably more than this to the total spectrum. However, other samples which do not show the presence of these proteins have sufficiently low total protein/DNA ratios to allow the residual non-histone contribution to be discerned. That they do not could be due to some nonlinearity in the contribution of different components in some of the samples. On the other hand, it may indicate that the residual non-histone proteins are artifact; the result of protein denaturation at higher salt.

Equation 3, using the above determined values (Figures 4 and 6), fits the experimental data well. The standard deviation of the fit from the experimental spectra at all the fitted wavelengths averages 3% of the signal at that wavelength.

The CD spectra of chromosomal salt-soluble proteins and residual non-histone proteins are indicative of the two protein classes having different average conformational properties. Some information about their conformations can be obtained by representing the spectra in Figures 4 and 6 by a linear model,

$$[\theta]^{\lambda}_{Prot} = f_{\alpha}[\theta]^{\lambda}_{\alpha} + f_{\beta}[\theta]^{\lambda}_{\beta} + f_{\gamma}[\theta]^{\lambda}_{\gamma} \quad (4)$$

in which only distinct spectra for helix, $[\theta]^{\lambda}_{\alpha}$ β form, $[\theta]^{\lambda}_{\beta}$, and unordered coil, $[\theta]^{\lambda}_{\gamma}$, are assumed to contribute to the CD of proteins by the fraction of peptide present in each of these conformations (the fractions are given respectively as $f_{\alpha}, f_{\beta}, f_{\gamma}$). The f_i are solved by least squares using a suitable set (bases set) of reference CD spectra, $[\theta]^{\lambda}$. Here, two bases sets are used, and the results are given in Table II. When a bases set derived from globular proteins in the solid state (Chen et al., 1972) is used, the salt-soluble chromatin proteins are seen to have twice as much helical content and about the same amount of β form and random coil as the residual non-histone proteins. Use of a set of $[\theta]^{\lambda}$ calculated from poly-L-lysine in solution (Greenfield and Fasman, 1969) gives similar results, except that more β form content is seen. The square roots of the variances of the least-squares solutions indicate that the set of reference CD derived from globular proteins can be fit somewhat better to the data than the poly-L-lysine reference set (Table II).

Table I: Calculated Protein Content of Chromatins and Nucleoproteins.[a]

Source	Sample						
	CHR[b]	NP LS	NP 0.6	NP 1.0	NP 1.2	NP 3.0	
Chick	3.3 ± 0.3	2.1 ± 0.3	1.8 ± 0.3	1.4 ± 0.2	1.0 ± 0.2	0.3 ± 0.1	Measured P/D[c]
embryo	3.3	2.9	1.7	1.3	1.0	0.3	Calcd P/D[d]
brain	0.0	0.0	0.0	0.1	0.4	0.3	Calcd R/D[e]
Pig	3.1 ± 0.3	2.6 ± 0.2	2.1 ± 0.2	1.8 ± 0.2	1.4 ± 0.2	0.8 ± 0.1	
cerebellum	2.9	2.8	2.2	1.8	1.3	0.8	
	0.0	0.0	0.0	0.0	0.4	0.6	
Myeloma	2.3 ± 0.4	1.9	1.5 ± 0.1	1.0 ± 0.1	0.7 ± 0.1	0.2	
	2.4		1.7	0.9	0.7	0.3	
	0.0		0.0	0.5	0.2	0.3	
Calf	1.9 ± 0.3	1.9	1.4 ± 0.2	0.9 ± 0.1	0.9 ± 0.1	0.3 ± 0.1	
thymus	2.2		1.7	1.1	0.9	0.3	
	0.0		0.0	0.0	0.0	0.3	
Chicken	2.2 ± 0.1		1.7 ± 0.1	1.2 ± 0.3	0.8 ± 0.1	0.2 ± 0.1	
erythrocyte	2.0		1.6	1.1	0.8		
	0.0		0.0	0.0	0.4		

[a] Calculated by least-squares fit of the experimentally determined CD spectra with spectra calculated for DNA, salt-soluble chromosomal proteins, and residual non-histone proteins. [b] Chromatin. Other abbreviations: NPLS, nucleoprotein low salt; NP 0.6, NP 1.0, NP 1.2, and NP 3.0, nucleoproteins produced by dissociation of chromatin with NaCl–5 m*M* phosphate solutions of ionic strengths 0.6, 1.0, 1.2 and 3.0, respectively. [c] Experimentally determined protein/DNA mass ratio. [d] Calculated protein/DNA mass ratio. [e] Calculated residual non-histone protein/DNA mass ratio.

Table II: Fraction Helix, β Form, and Unordered Coil by Least-Squares Analysis of Spectra Data.

Bases set	Protein	Helix	Fraction β Form	Unordered
Chen et al., 1973	Residual non-histone proteins	0.09 ± 0.01	0.11 ± 0.02	0.80 ± 0.02
	Salt-soluble proteins	0.18 ± 0.01	0.11 ± 0.01	0.71 ± 0.01
Greenfield and Fasman, 1969	Residual non-histone proteins	0.06 ± 0.02	0.35 ± 0.06	0.58 ± 0.07
	Salt-soluble proteins	0.18 ± 0.02	0.24 ± 0.04	0.57 ± 0.04

Discussion

Chromatins isolated from tissues differing in nuclear synthetic activities and which vary markedly in protein (Table I) and RNA content (Hjelm and Huang, 1975) have very similar CD spectra between 200 and 250 nm (Figure 1). Removal of 25–50% of the chromosomal proteins (histone I and as much as 50% of the non-histone proteins, Hjelm and Huang, 1974) leaves the spectra between 200 and 235 nm unaltered (Figure 2). With removal of larger amounts of protein, the CD bands below 235 nm are seen to become less negative; the resulting spectra are different for each chromatin (Figure 2). We have shown previously (Hjelm and Huang, 1974) that little or no protein degradation has occurred in these samples.

The spectra of the various chromatins and depleted nucleoproteins are resolved into three components: contributions are seen from the salt-extractable proteins (Figure 4), the residual non-histones, and DNA (Figure 5) (eq 3). The three spectral terms are derived from the two-step linear relationship between the spectral ratio, $\theta^{\lambda}/\theta^{250}$, and the protein/DNA molar ratio of the samples (Figure 3 and 5).

Equation 3 implies that the three molar ellipticities in the right side of the equation are constant; thus within the error of these experiments, the average CD spectra between 200 and 250 nm of DNA, $[\theta]^{\lambda}_{DNA}$, salt-soluble proteins, $[\theta]^{\lambda}_{s}$, and residual non-histone proteins, $[\theta]^{\lambda}_{r}$, are the same in all samples, regardless of the source or composition of the chromatin or nucleoproteins.

While the manuscript of this work was in preparation, a similar analysis as given here appeared (Hanlon et al., 1974). It indicates that the CD below 250 nm of calf thymus chromatin can be resolved into three protein spectra and a DNA spectrum assuming the CD below 250 nm of DNA in chromatin to be the same as that of DNA at low ionic strength. Their work deviates from the present study in that we find no evidence for any more than two protein components. Hanlon et al. (1974) separate the partially depleted nucleoproteins from the dissociated material by ultracentrifugation after the chromatin is exposed to either NaCl or sodium deoxycholate (Bartley and Chalkley, 1972). In this paper gel filtration is used to purify the partially depleted nucleoproteins (Hjelm and Huang, 1974).

The large contribution of proteins to the CD of chromatin between 200 and 250 nm has prevented the elucidation of CD of DNA in this region. These results show that integration of DNA into chromatin does not alter the CD of DNA below 250 nm; thus the spectrum calculated and shown in Figure 6 is identical with that of DNA free in solution at low ionic strength (Figure 2), and corresponds to the CD of the DNA in chromatin. This is a direct result of the invariance of $[\theta]^{\lambda}_{DNA}$ with protein content of chromatin and depleted nucleoproteins. This part of the DNA spectrum is, therefore, unlike the positive CD region above 250 nm. In chromatin the positive band is depressed to about 50% of its value for DNA in solution at low ionic strength (Shih and Fasman, 1970). That the near 250 nm spectrum is more sensitive to the conformation of DNA is consistent with observations of DNA in concentrated solutions of alkyl chlorides (Studdert et al., 1972; Ivanov et al., 1973) and at low temperatures (Studdert et al., 1972). Under these con-

Table III: Non-histone to DNA and Histone to DNA Mass Ratios of Chromatins and Nucleoproteins.[a]

		CHR[b]	NP Low Salt	NP 0.6	NP 1.0	NP 1.2	NP 3.0
Chick	NHP/D	2.0–2.3	0.8–1.1	0.8–1.0	1.9–1.1	0.7–0.8	0.3
embryo brain	H/D	1.3–1.0	1.3–1.0	0.9–0.75	0.5–0.4	0.3–0.2	0.0
Pig	NHP/D	1.8–2.1	1.3–1.6	1.1–1.3	1.2–1.4	1.0–1.1	0.8
cerebellum	H/D	1.3–1.0	1.3–1.0	0.9–0.75	0.6–0.4	0.4–0.3	0.0
Myeloma	NHP/D	1.0–1.3	0.6–0.9				0.2
	H/D	1.3–1.0	1.3–1.0				0.0
Calf	NHP/D	0.6–0.9		0.4–0.6	0.6	0.6	0.3
thymus	H/D	1.3–1.0		0.9–0.75	0.3	0.2	0.0
Chicken	NHP/D	0.9–1.2		0.7–0.9			0.2
erythrocyte	H/D	1.3–1.0		0.8–0.6			0.0

[a] Calculated from a previous paper (Hjelm and Huang, 1974). The first line under each sample is the non-histone/DNA mass ratio, NHP/D, and the second line is the histone/DNA mass ratio, H/D. Range of values assume respectively a chromatin histone/DNA mass ratio of 1.3 and 1.0. [b] Abbreviations are the same as in Table I.

ditions the CD of DNA above 250 nm is observed to be depressed; below this wavelength the spectrum is unaltered. The observation of other workers (Permogarov et al., 1970; Henson and Walker, 1970) that the CD of DNA in chromatin above 250 nm is like that of DNA in aqueous solutions containing high concentrations of NaCl, KCl, etc. can now be extended to the region between 200 and 250 nm. Therefore, over its entire measurable range, the CD of DNA in chromatin is intermediate between the CD spectra of DNA in B and C forms, as measured in DNA films at different relative humidities (Tunis-Schneider and Maestre, 1970). The CD spectrum derived for the salt-soluble proteins is an average of the histones and the salt-extractable non-histone proteins. The chromatin, nucleoprotein low salt, and nucleoprotein 0.6 samples, which demonstrate indistinguishable CD when expressed as deg cm^2/dmol of protein (Figures 1 and 2), differ considerably in the fraction histone constituting the total salt-soluble proteins of the samples (Table III). For example, the histone/salt-soluble non-histone protein mass ratio is 1/3 in chick embryo brain chromatin and 3/4 in calf thymus nucleoprotein 0.6 (Table III). This implies that the spectra of histones and the salt-extractable non-histone proteins are very similar. Furthermore, there is little difference in the content of non-histone proteins between samples extracted with solutions of low ionic strength (0.2–0.35) and samples extracted with solutions of ionic strength 1.2 (Table III). Therefore, in Figure 3 the region between protein/DNA molar ratios of 2 and 5 represents only a difference in histone content; thus the slopes of the lines in this region give the CD of the histone proteins. It is clear from Figure 3 that the slopes do not differ significantly from the slopes of the lines in the area which represents a variation in only the non-histone proteins (above protein/DNA molar ratio of 5.5). Again, the CD spectrum for salt-soluble proteins in chromatin given in Figure 4 is a good approximation for both histones and salt-soluble non-histone proteins.

It would be interesting to draw some conclusions as to the likely CD spectra of the individual histone fractions from the nucleoprotein 1.0 and nucleoprotein 1.2 samples. The data indicate that the individual histone fractions have similar CD. However, the data are not sufficiently precise to determine the extent to which the individual histone fractions may be similar or different.

We have calculated that the CD of the salt-extractable proteins of chromatin is consistent with 18% of the amino acid residues being incorporated into segments of proteins that are helical. This figure is precisely that calculated by Oriel (1966) from the ORD of chromatin, and close to the value, 20%, calculated from the CD of chromatin (Henson and Walker, 1970). The similarity of our values with those previously calculated is due to the fact—demonstrated in this work—that the CD in the region below 235 nm is dominated by the salt-extractable proteins. The fraction of total protein calculated to be in β form varies with the basis set (CD of helix, β form, and unordered coil) used to make the estimate, but indicated the amount to be substantial (Table II). Infrared and X-ray studies, on the other hand, do not indicate the presence of β form in proteins in chromatin (Bradbury and Crane-Robinson, 1971). However, the conformation of protein is only detectable by these techniques if several sheets of hydrogen-bonded protein chains are present (Bradbury and Crane-Robinson, 1971). This disagreement points out a limitation in the technique used here to estimate the quantitative presence of each conformation in that more than three bases spectra may be necessary to reconstruct the CD of protein below 250 nm (Chen et al., 1972, 1974). This is in addition to the important question of the appropriateness to this system of the bases sets used. Thus the numbers on Table II are rough estimates. Regardless, the estimates do indicate that the salt-soluble proteins in chromatin—including histones—have conformations much like globular proteins.

That the total histone complement in chromatin should have a CD spectrum compatible with considerable secondary structure (Figure 4) is consistent with earlier observations on the CD in this region of chromatin and isolated histones. ORD studies of total histone in solution at very low ionic strength demonstrate mostly random coil (Bradbury et al., 1965; Jirgensons and Hnilica, 1965), but take on considerable secondary structure at higher ionic strengths (0.1–1.0 *M* NaCl). CD studies verify this result and show that the sum of CD spectra histone and DNA between 200 and 250 nm in solution at very low ionic strength is not like the CD spectrum of chromatin. The sum of the two spectra in 2.0 *M* NaCl is very similar to the spectrum of chromatin between 200 and 250 nm (Permogarov et al., 1970; Fric and Sponar, 1971; Ramm et al., 1972); thus the interactions of histones and DNA result in conformations which are reminiscent of those occurring in solutions of concentrated NaCl (Permogarov et al., 1970; Fric and Sponar, 1971; Ramm et al., 1972). This does not necessarily mean that the conformations are the same in both systems; this applies especially to the proteins.

The linear regression lines for the nucleoprotein 3.0 samples (Figure 5) show that the CD of these depleted nucleo-

proteins can be reconstructed from a single protein spectrum (Figure 6) and a spectrum for DNA (Figure 6). A demonstration of this is the ability of eq 3 to predict the known residual non-histone/DNA ratios in each of the nucleoprotein 3.0 samples. Furthermore, when the calculated ratios are placed back into eq 3, the calculated spectrum is within 3% of the values for the measured CD. As with the CD for the salt-soluble proteins, the spectrum for the residual non-histone proteins represents an average. The form of the spectrum indicates that these proteins are more extended in conformation than the salt-extractable proteins may be related to special structural requirements for their interaction with DNA, or to their function. Again, we cannot rule out the possibility that they are an artifact of NaCl denaturation.

It is possible that the spread in the protein/DNA molar ratios of the nucleoprotein 3.0 samples used to determine the least-squares lines in Figure 5 is the result of inaccuracy in the protein and DNA assays used, and is not the result of any real variation in residual non-histone protein content. Although the differences in protein/DNA ratios are certainly real between nucleoprotein 3.0 from pig cerebellum and nucleoprotein 3.0 from other sources, it is not possible to determine to what extent the differences among the other samples are also real; particularly in cases where the nucleoprotein 3.0 samples are from the same source. This might cast doubt on the assertions of the preceding paragraph except, as stated, *all* the nucleoprotein 3.0 CD spectra are reconstructable with a single protein and a single DNA spectrum. The assertions of the above paragraph are also supported by the fact that the least-squares lines in Figure 5 when extrapolated to zero protein content give a derived CD spectrum which is indistinguishable (Figure 6) from the measured spectrum of DNA (Figure 2B).

Although many studies have appeared on non-histone proteins, very little is known about the 3.0 *M* NaCl residual proteins. Most "residual" non-histone proteins described in the literature are proteins remaining after dilute acid or salt extraction of the nuclei; thus they may not be identical with the proteins described here.

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