The Contribution of RNA and Non-Histone Proteins to the Circular Dichroism Spectrum of Chromatin†

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ABSTRACT: This paper is an investigation of the contribution of low salt extractable RNA and non-histone proteins to the circular dichroism of chromatin. Circular dichroism (CD) of chromatin above 250 nm is due mainly to DNA and is different from that of DNA free in solution. In addition, to a smaller extent, we find that low salt extractable RNA and/or non-histone protein side chain chromophores contribute significantly to the spectra in this region and account for the major differences observed among the CD spectra of chromatins isolated from the five tissues studied: pig cerebellum, myeloma, calf thymus, chick embryo brain, and chick erythrocytes.

Studies on isolated chromatin find it to consist of DNA, protein, and small amounts of RNA (Bonner et al., 1968; Stellwagen and Cole, 1969; Hearst and Botchan, 1970; Georgiev, 1969; DeLange and Smith, 1971; Elgin et al., 1971; Huang and Hjelm, 1974). The proteins consist of histones, a discrete group of highly basic, small proteins which are intimately associated with the DNA; and the non-histone proteins, a class of highly heterogeneous proteins which includes enzymes, structural proteins, and genetic regulators. Determination of the interaction and conformation of chromatin components is a problem of great importance. Its resolution is key to an understanding of the basis of chromosome structure and function in higher plants and animals.

This and two related papers (Hjelm and Huang, 1974a,b) deal with the circular dichroism (CD) of chromatins isolated from tissues differing in nuclear synthetic activities. Of these, three produce RNA and DNA, calf thymus, 11-day-old chick embryo brain, and myeloma K41 (mucine solid tumor); one, pig cerebellum—like other adult nervous tissues (Deluca et al., 1953; Bendick et al., 1953; Leblond and Walker, 1956; Koenig, 1958—produces only RNA; and one, chicken erythrocyte, is completely repressed in nuclear synthetic activity (Williams, 1971; Neelin et al., 1964). In addition, the in situ nucleochromatin of avian nucleated erythrocytes is completely condensed (Vidali et al., 1973), unlike the nucleochromatins of the other tissues studied. These chromatins also differ in the relative amounts of the various chromatin components; specifically non-histone proteins and RNA.

In our initial observations on some of these chromatins we noted differences among their CD in the positive ellipticity region above 260 nm (Hjelm and Huang, 1972). Similar variations have recently been reported by Lin et al. (1974) in chromatins isolated from normal diploid and SV40 transformed human fibroblasts. These differences are either due to variations in the secondary structure of chromatin DNA, or to the trivial contribution of RNA (as suggested by Simpson and Sober, 1970) and/or protein side-chain chromophores. Resolution of this question has considerable intrinsic value and is important to our studies on the roles of histone and non-histone proteins in determining the secondary structure of DNA in chromatin (Hjelm and Huang, 1974a). In addition, a study of the possible contribution of other chromophores besides DNA to this region may give information on the structure of the RNA and the cystine and aromatic-containing proteins in chromatin.

In this paper we report that the RNA and/or protein side-chain chromophores contribute significantly to the spectrum above 260 nm of chromatins; this contribution accounts for the major differences observed in the CD of chromatins in this region of the spectrum.

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 a 10% (w/v) solution of trisodium citrate (100 ml of solution/1000 ml of blood; Murray et al., 1968). Myeloma tumors are cultured in vivo in Balb/c mice, and are provided by the courtesy of Mrs. Susan Ayang and Dr. Janet Stavnezer. Chick brains are obtained from 11-day-old embryos. All materials are used immediately after obtention, with the exception of calf thymus which is often stored at −20° until use.

Methods

Isolation of Nuclei. Pig cerebellum and chick embryo nuclei are isolated by the method of Shaw and Huang (1970). Nuclei from chicken erythrocytes are prepared according to the procedure outlined by Murray et al. (1968). Nuclei from myeloma are prepared by the method of E. Stavnezer (personal communication). According to this procedure, 4–5 g of minced tissue is made into a 10% homogenate (w/v) in 0.32 *M* sucrose, 5 m*M* $MgCl_2$, 10 m*M* Tris (pH 7.9), and 0.5% Triton X-100 (homogenization medium) by ten passes in a Teflon-glass homogenizer. The homogenate is filtered through eight layers of cheesecloth,

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then centrifuged at 3500 rpm for 15 min in a Sorvall SS-34 rotor. The pellet is resuspended into fresh homogenization medium without Triton X-100 (1 ml/g of starting material). To this mixture is added three volumes of 2.3 *M* sucrose, 5 m*M* $MgCl_2$, and 10 m*M* Tris (pH 7.9). The suspension is centrifuged at 16000 rpm for 45 min in an SS-34 rotor. The pellet contains the purified nuclei.

Preparation of Chromatin. Chromatin is isolated by the method of Huang and Huang (1969). Except for calf thymus, where finely chopped whole tissue is used, the starting material is isolated nuclei (Shaw and Huang, 1970). Chromatin isolation and all subsequent procedures are done at 4°, unless otherwise noted.

Histone degradation can occur during the isolation of calf thymus chromatin, but can be prevented by the addition of sodium bisulfite (0.05 *M*, pH 8.0) to the saline–EDTA (Bartley and Chalkley, 1970). No histone degradation is observed in the other chromatins, and the standard saline–EDTA solution is used in these cases.

Low Salt Extraction of Chromatin. Chromatin is extracted in solutions of saline–EDTA or of 5 m*M* phosphate and NaCl ranging in ionic strength from 0.2 to 0.35. To unsheared chromatin (15–20 OD_{260}/ml) ice-cold 0.2*M* sodium phosphate (pH 6.8) is slowly added with constant stirring, until a concentration of 5 m*M* is reached. Cold 5 *M* NaCl is then added in the same manner until the chromatin suspension is at the desired ionic strength. During this procedure the chromatin is kept cold in an ice bucket. The precipitated chromatin is stirred for a few minutes, then pelleted at 5000 rpm (SS-34 rotor) for 10 min. The pellets are made into nucleoprotein gels by swelling into solutions of decreasing Tris buffer (pH 8.0) as in the chromatin isolation procedure. The extracted chromatin is termed nucleoprotein low salt. The extract containing RNA and non-histone proteins and some DNA is termed low salt extract.

Quantitative Determination of Chromatin Components. Protein determinations are made by the method of Lowry et al. (1951) and are made directly on the various chromatins and depleted nucleoproteins. Determination of the RNA content of each sample is done by first hydrolyzing the sample with 0.3 *M* KOH for 18 hr at 37°, followed by the precipitation of the KOH, unhydrolyzed DNA, and protein with 8% perchloric acid. The amount of soluble ribonucleotides is then determined by the orcinol reaction (Schneider, 1957). Diphenylamines (Burton, 1956) for DNA are made after hydrolysis in hot 5% perchloric acid (70°, 10 min).

Isolation of RNA for Electrophoresis and Circular Dichroism. RNA is extracted from the low salt extract with buffer-saturated phenol containing 0.5% sodium dodecyl sulfate (SDS).[1] The phenol-extracted RNA is dissolved in 0.01 *M* sodium acetate (pH 4.7) and applied to a small DEAE-cellulose column equilibrated in the same buffer. The column is then washed with 0.3 *M* NaCl, 10 m*M* $MgCl_2$, and 0.01 *M* sodium acetate (pH 4.7). Elution of low molecular weight RNA from the column is effected by 1.0 *M* NaCl. Sixty percent of the OD_{260} loaded on the column is in this fraction. The RNA is dialyzed against 10^{-2} *M* NaCl for CD and against distilled water for acrylamide gel electrophoresis.

Analysis of Chromosomal Proteins. Chromosomal proteins are analyzed by SDS and urea acrylamide gel electrophoresis. Low salt extract samples are dialyzed against distilled water, then lyophilized. Total chromosomal proteins and proteins from nucleoprotein low salt samples are dissociated from DNA by 7 *M* urea–2 *M* NaCl (Shaw and Huang, 1970), followed by centrifugation at 30,000 rpm for 36 hr at 4° in a Spinco 30 rotor. The protein-containing supernatant is dialyzed and lyophilized. The dried samples are dissolved in either 4 *M* urea–0.9 *M* acetic acid, or 0.1% SDS. Samples are reduced by incubation in 0.1 *M* β-mercaptoethanol at 37° for 1 hr. The samples in urea–acetic acid are applied to 15% polyacrylamide–urea gels (after Panyim and Chalkley, 1969), and electrophoresed at 100 V for 4 hr. The gels are stained with 0.1% Buffalo Blue-Black (in 7% acetic acid) for 4 hr. Destaining is carried out by diffusion into 7% acetic acid. Samples dissolved in SDS are applied to 8.75% polyacrylamide–SDS gels (after Laemmli, 1970). Electrophoresis through the running gel is done at 2 mA/gel. Bromophenol Blue is used as a marker. The SDS gels are stained and destained according to the protocol outlined by Fairbanks et al. (1971); Coomassie Brilliant Blue is used to stain the proteins in this procedure.

Measurement of Circular Dichroism. Chromatins and depleted nucleoproteins are sheared (Waring Blendor, 80 V variac 1 min) then dialyzed against 0.7 m*M* sodium phosphate (pH 6.8). Samples of low salt extract are measured in the solutions in which they are extracted. Concentrations of chromatin or nucleoproteins are adjusted to between 1.0 and 1.7 OD at 260 nm. Unless otherwise stated, the CD spectra are measured at 4°. CD spectra are recorded on a Cary 60 spectropolarimeter fitted with a 6003 CD attachment; 10-mm path-length cells are used.

Uv spectra are measured by a Cary 14 spectrometer or a Beckman DU spectrometer.

Calculations. CD data are collected at intervals of 2.5 nm from the chart readout of the Cary 60. The data are reported as molar ellipticity [(deg cm^2)/dmol] and are calculated from the data by the equation: $[\theta] = (M/10cl)\theta$, where $[\theta]$ is the molar ellipticity, M is the average molecular weight of a nucleotide or amino acid residue, c is the concentration of DNA, RNA, or protein in g/ml, l is the path length of the cell in centimeters, and θ is the measured ellipticity of the sample. M is 309 for DNA, 327 for RNA, and 115 for protein.

All data are presented plus and minus standard deviation. Reduction of the CD data is carried out by Basic and Fortran II programs run on a Hewlett-Packard 2100 computer.

Results and Discussion

Circular Dichroism above 250 nm of Chromatins. Consistent with the observations of Shih and Fasman (1970) the CD spectra above 250 nm of the various chromatins are different from that of DNA (Figure 1). It is also apparent that the CD of the various chromatins are different in some cases, especially in the region about 273 nm where chromatins isolated from pig cerebellum, chick embryo brain, and myeloma have a peak (Figure 1) of about 6000 (deg cm^2)/dmol of DNA (Table I); chicken erythrocyte and calf thymus chromatin demonstrate only a shoulder in this region (Figure 1), 4000–5000 (deg cm^2)/dmol of DNA in amplitude (Table I).

The spectral differences between the two sets of chromatins are better shown by the ratio of ellipticities at 273 and 283 nm (Table I). Pig cerebellum, chick embryo brain, and myeloma chromatins have 273/283 ratio (about 1.16) which is significantly different from the ratio (approximately 1.0) for chicken erythrocyte and calf thymus chromatins.

[1] Abbreviation used is: SDS, sodium dodecyl sulfate.

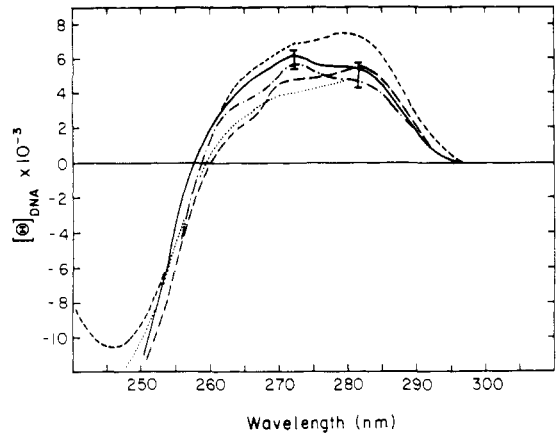

FIGURE 1: Circular dichroic spectra of various chromatins. (—) Chick embryo brain; (— · — ·) pig cerebellum and myeloma; (- -) chicken erythrocyte; (· · ·) calf thymus; (- - -) calf thymus DNA. The spectra are averages of several determinations as indicated in Table II, but retain the characteristics of the individual spectra. Molar ellipticities are expressed as (deg cm^2)/dmol of DNA.

Table I: Ellipticities at 273 nm and 283 nm of Chromatins and Nucleoproteins and Their Spectral Ratios.

	Chromatin		NP Low Salt[a]	
Source	273 nm	283 nm	273 nm	283 nm
Chick embryo brain	6220 ± 620	5100 ± 480	4400 ± 560	4550 ± 490
	1.19 ± 0.09[b]		0.96 ± 0.05[b]	
	(18)[c]		(18)	
Pig cerebellum	5800 ± 470	4670 ± 480	4740 ± 600	4270 ± 480
	1.16 ± 0.08[b]		1.08 ± 0.08[b]	
	(17)		(17)	
Myeloma	5750 ± 630	4620 ± 660	3898	4455
	1.15 ± 0.03[b]		0.87[b]	
	(9)		(1)	
Calf thymus	4000 ± 480	4650 ± 610	3850	4275
	0.93 + 0.08[b]		0.90[b]	
	(7)		(1)	
Chicken erythrocyte	4790 ± 290	5310 ± 570		
	0.86 ± 0.08[b]			
	(6)			

[a] Nucleoprotein low salt (see Methods). [b] Ratio of ellipticities at 273 and 283 nm. [c] Number of determinations given in parentheses.

Table II: Protein to DNA Mass Ratios of Chromatins and Chromatins Dissociated by Solutions of Low Ionic Strength.

Source	Chromatin	NP Low Salt
Chick embryo brain		
Protein/DNA	3.3 ± 0.3	2.1 ± 0.3
Fraction of protein[a]	1.0	0.66 ± 0.07
No. of determinations	(17)	(6)
Pig cerebellum		
Protein/DNA	3.1 ± 0.3	2.6 ± 0.2
Fraction of protein	1.0	0.86 ± 0.04
No. of determinations	(17)	(12)
Myeloma		
Protein/DNA	2.3 ± 0.4	1.9
Fraction of protein	1.0	0.79
No. of determinations	(9)	(1)
Calf thymus		
Protein/DNA	1.9 ± 0.3	1.9
Fraction of protein	1.0	0.86
No. of determinations	(12)	(1)
Chick erythrocyte		
Protein/DNA	2.2 ± 0.1	
Fraction of protein	1.0	
No. of determinations	(7)	

[a] The fraction of protein remaining on each sample relative to that of the original chromatin sample.

Table III: RNA to DNA Mass Ratio of Salt-Extracted Chromatins.[a]

Source	CHR[b]	Sample NP LS[c]	P LS[d]
Chick embryo brain	0.102 ± 0.020	0.064 ± 0.016	0.043 ± 0.006
	(7)[e]	(10)	(8)
Pig cerebellum	0.122 ± 0.020	0.084 ± 0.034	
	(9)	(13)	
Myeloma	0.120 ± 0.040	0.080 (1)	
	(3)		
Calf thymus	0.044 ± 0.008	0.030 (1)	
	(3)		
Chicken erythrocyte	0.030 (1)		

[a] RNA determined by the orcinol reaction (Schneider, 1957); DNA determined by the diphenylamine procedure (Burton, 1956). [b] Chromatin. [c] Nucleoprotein low salt (see Methods). [d] Low salt extract. The ratio given here is the amount of RNA in the extract relative to the total DNA in the chromatin sample from which the extraction is made. [e] Number of determinations given in parentheses.

The difference is significant to greater than 0.99 by Student's *t* test.

Chromatin, like other macromolecular aggregates, scatters light. This can result in CD artifacts (Gordon, 1972; Dorman and Meastre, 1973; Holzwarth et al., 1974), and could account for the observed CD differences between the chromatins. However, all the chromatins have about the same turbidity; the OD_{320}/OD_{260} ratio is about 0.05 in most cases. Turbidity of the samples with large amounts of protein can vary a great deal, depending on handling; yet we find no detectable variation in the CD about 283 nm. The spectra are noisier when the samples are turbid, but it appears that this effect does not account for the differences observed.

Composition of the Chromatins Studied. The differences observed in the CD about 273 nm of the various chromatins may reflect a variation in DNA secondary structure with different RNA transcription activity, or they may reflect the presence of protein side-chain or RNA chromophores; thus a chemical analysis of the chromatins is necessary.

The amounts of RNA and protein relative to DNA (μg/μg) for the various chromatins are given in Tables II and III. The RNA content is greatest in those chromatins isolated from tissues having the highest capacities for RNA synthesis (Table III); these are also the samples demonstrating the higher ellipticities about 273 nm (Figure 1, Table I). A similar correlation appears to be present for protein content, with the exception of myeloma chromatin (Table II). Studies on the histone content of chromatins from a wide variety of plants and animals reveal that the histone/DNA mass ratio is close to 1:1 in all cases (Bonner et al., 1968); thus the differences observed in protein content of these samples must be due to non-histone proteins. Positive correlations between nuclear activity and the presence of large amounts of non-histones and RNA have been observed by other workers (Dingman and Sporn, 1964; Marushige and Dixon, 1969; Marushige and Ozaki, 1967).

Low-Salt Extraction of Chromatins: Quantitative and Qualitative Analysis. Solutions of NaCl–5 m*M* phosphate (pH 6.8) of ionic strength between 0.2 and 0.35 extract a substantial amount of proteins and RNA from pig cerebellum, chick embryo brain, and myeloma chromatins (Tables

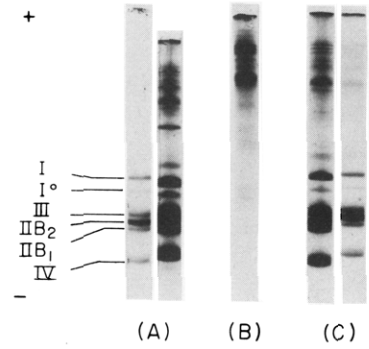

FIGURE 2: Urea-polyacrylamide gel electrophoresis of proteins from chicken embryo brain chromatin and low salt extractable chromatin. (A) Chromatin; (B) proteins in low salt extract; (C) proteins remaining in nucleoprotein low salt. Dual gels of the same sample are loaded with different amounts of total proteins. Direction of electrophoresis is positive to negative.

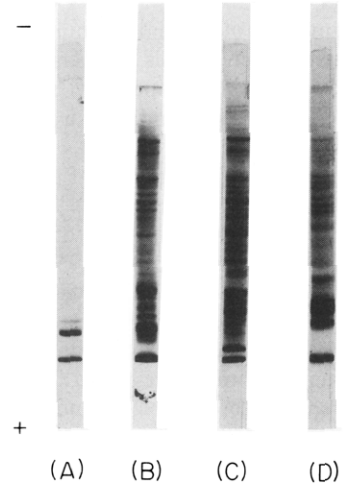

FIGURE 3: SDS-polyacrylamide gel electrophoresis of proteins from chick embryo brain chromatin and low salt extracted chromatin. (A) Total histones; (B) chromatin protein; (C) proteins removed by low salt; (D) proteins remaining on nucleoprotein low salt. The bottommost band is the buffer front. The direction of electrophoresis is negative to positive.

II and III. About 50% of the RNA and 20–30% of the protein are removed from these chromatins by extraction with these solutions; however, pig cerebellum is quite variable in the amount of RNA removed (Tables III and IV). Calf thymus chromatin may suffer some protein loss from this treatment (Table II), but no significant loss of RNA is apparent (Table III).

The nature of the protein removed by extraction with low salt is indicated by SDS- and urea-polyacrylamide gel electrophoresis (Figures 2 and 3). Low salt extraction of chick embryo brain or pig cerebellum chromatin does not remove detectable amounts of histone proteins. Polyacrylamide gels of extracted proteins from chick embryo brain are shown as an example (Figure 2): no bands are present in the histone regions of the gels. Low salt solutions do not remove all the non-histone proteins: SDS-polyacrylamide gels of chick embryo brain samples show that the non-histone proteins removed are from the entire molecular weight range of these proteins (Figure 3).

The RNA removed from chick embryo brain chromatin is found by sucrose gradient centrifugation to be smaller than 18 S. Acrylamide gels (after Loening, 1968) show the RNA to consist of discrete, low molecular weight species,

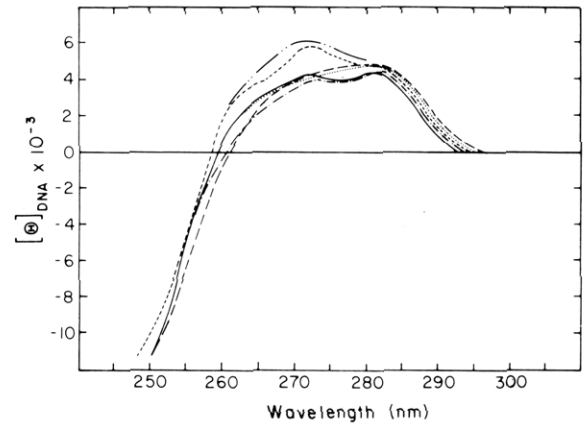

FIGURE 4: Circular dichroism of chromatin extracted with solutions of low ionic strength. (— • • —) Chick embryo brain chromatin; (- - - -) pig cerebellum and myeloma chromatin; (• • • •) calf thymus chromatin and calf thymus nucleoprotein low salt; (—) pig cerebellum nucleoprotein low salt; (- • - •) myeloma nucleoprotein low salt; (- -) chick embryo brain nucleoprotein low salt.

Table IV: Content of Material Removed from Chromatin by Solutions of Low Ionic Strength.

Sample	Mass Ratios (μg/μg)	
	DNA/RNA	Protein/RNA
Pig cerebellum	1.02 ± 0.12 (4)[a]	47 ± 4 (5)
Chick embryo brain	0.25 ± 0.16 (9)	23 ± 6 (9)

[a] Number of determinations given in parentheses.

ranging in size from 4 S to 11 S.

Changes in the Circular Dichroism of the Chromatins with Low-Salt Extraction. The effects of low salt (ionic strength of 0.2–0.35) extraction on the CD of the chromatins are shown in Figure 4 and Table I. Low salt extraction causes a considerable attenuation of the 273-nm peak in all chromatins originally demonstrating it: pig cerebellum, chick embryo brain, and myeloma (Figure 1, Table I). Pig cerebellum chromatin shows the least attenuation (Table I), and perhaps this is due to the relative difficulty in removing RNA from this material (Tables III and IV). The CD spectra of the nucleoprotein low salt samples from these sources are now fairly similar to one another and to that of calf thymus chromatin (Figure 4, Table I). Calf thymus chromatin, on the other hand, is not significantly affected by low salt extraction. The ratios of sample ellipticities at 273 and 283 nm (Table I) verify that the shapes of the spectra for the three chromatins from pig cerebellum, chick embryo brain, and myeloma are indeed changed; whereas that from calf thymus is not. The spectra are effected by all salt concentrations between 0.2 and 0.35 *M* which remove non-histone and RNA.

Circular Dichroism of Low Salt Extracts. With the above information on the constitution and CD of the different chromatins and nucleoprotein low salts it is possible to address the major question of this paper: what are the causes of the differences observed in the CD spectra of these chromatins? Two alternatives exist: either the material removed from chromatin by low salt possesses a sufficient CD signal to account for the observed differences; or interactions are present in some chromatins—changing the CD which are destroyed by low salt extraction.

The contribution of the low salt extractable material to

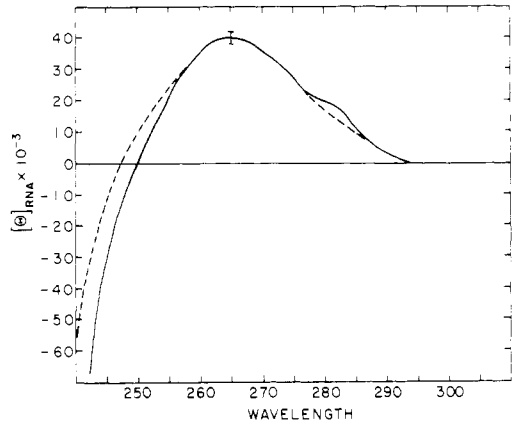

FIGURE 5: Circular dichroism of low salt extracts from chick embryo brain and pig cerebellum chromatins. (—) Pig cerebellum; (- - - -) chick embryo brain.

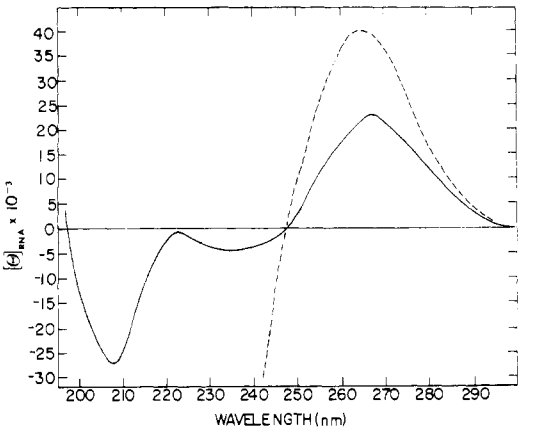

FIGURE 6: circular dichroism of low salt extract and RNA isolated from the extract of chick embryo chromatin. (—) RNA in 10^{-2} *M* NaCl; (- - - -) low salt extract in 0.3 *M* NaCl.

the CD above 250 nm of pig cerebellum and chick embryo brain chromatins is explored by direct observation of the low salt extracts (Figure 5). Between 255 and 275 nm the spectra of the two samples are seen to be identical with a maximum at 265 nm of approximately 40,000 (deg cm^2)/dmol when normalized to RNA content. We demonstrate below that this signal is sufficient to explain the observed differences among the chromatins studied. However, we need to explore some important consequences of the data given in Figure 5 before considering in detail the contribution of the low salt extractable material to the CD of chromatin.

The CD spectra of the low salt extracts normalized to RNA content (Figure 5) have the remarkable characteristic that the observed ellipticity, if entirely due to RNA, is 30% greater than any reported for RNA, either isolated (Brahms and Mommaerts, 1964; Scott et al., 1968; Samejima et al., 1968; Wolffe et al., 1968; Gratzer and Richards, 1971), or in a ribonucleoprotein particle (Sarker et al., 1967; Cox et al., 1970; Kay et al., 1970; Isenberg et al., 1971). In comparison with the CD of RNA isolated from the chick embryo brain low salt extract (about 23,000 (deg cm^2)/dmol RNA at 267 nm; Figure 6) the ellipticity of the total extract is seen to be approximately 17,000 (deg cm^2)/dmol of RNA greater. The relatively high salt conditions in which the CD of the low salt extract are measured do tend to make the RNA potentially more double helical than the isolated RNA which is measured at low ionic strength, explaining the relative blue shift seen in the samples (Gratzer and Richards, 1971), but not the extreme increase in ellipticity. Therefore, if RNA is assumed to be the only contributor to the spectra of the low salt extracts, then some heretofore unobserved conformation of RNA is involved.

Alternatives exist in addition to an extremely high ellipticity of RNA in the low salt extracts. Clearly, in addition to RNA, chromophores of DNA and/or aromatic amino acids could contribute, if present. Chemical analysis of the low salt extracts of pig cerebellum and chick embryo brain chromatins (Table IV) shows the presence of large amounts of DNA in addition to protein and RNA. The analysis indicates, also, that the pig cerebellum samples contain four times as much DNA and twice as much protein relative to the amount of RNA extracted as those of chick embryo brain chromatin. The uv spectrum of the low salt extract chick embryo brain (Figure 7) indicates the presence of relatively large amounts of aromatic amino acids in the nonhistone proteins extracted. This is inferred from the maximum of the low salt extract spectrum at 267 nm as compared to the 260 nm maximum in the uv spectrum of RNA isolated from the extract.

Referring again to the CD spectra of the low salt extracts from pig cerebellum and chick embryo brain chromatins in Figure 5, another remarkable feature of the spectra is that they are identical between 255 and 275 nm. This observation in combination with the differences between the samples in protein/RNA and DNA/RNA mass ratios (Table IV) allow us to calculate the approximate contribution of DNA and protein chromophores if either do, in fact, contribute to the spectra between 255 and 275 nm. If DNA and/or protein are contributing to the spectra between 255 nm and 275 nm, then the identity of the pig and chick spectra normalized to RNA content dictates that contributions of protein and DNA within this region relative to that of RNA must be the same in both pig and chick samples. Assuming a contribution of 23,000° by the RNA to the CD of the low salt extracts, then using the DNA content of the chick samples as the lowest common denominator (Table IV), the signal of the DNA at 265 nm must be ([θ] RNA low salt extract − [θ] isolated RNA)C_{RNA}/C^{C}_{DNA} = 17,000 × 4 = 68,000 (deg cm^2)/dmol of DNA. This value is much higher than the highest reported values for the CD of DNA which are observed in such solvents as 80% aqueous dioxane at very low ionic strength (38,000 (deg cm^2)/dmol, Ivanov et al., 1973) and 80% ethanol in water (32,500 (deg cm^2)/dmol, Ivanov et al., 1973; Girod et al., 1973). Similar calculations for protein yield an ellipticity at 265 nm of +250 (deg cm^2)/dmol of protein. This is a reasonable magnitude for aromatic and cystine residue-containing proteins, though a positive value for the protein side chains in this region of the spectrum is unusual (see for example, Adler et al., 1973).

It should be noted that the spectra required for RNA and/or DNA to explain these results are essentially of the same position, sign, and shape as those observed for RNA (Gratzer and Richards, 1971) and DNA in the A form (Tunis-Schneider and Maestre, 1970); though, again, they differ considerably in magnitude.

From these considerations there is clearly no good reason to accept any one of these possibilities over any of the other two. Each requires invoking a CD spectra that has not been observed previously. However, if large contributions from

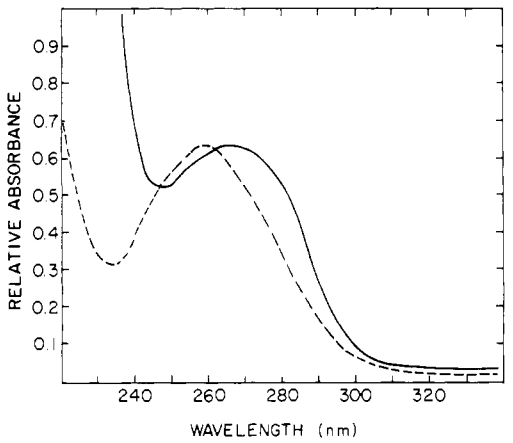

FIGURE 7: Uv spectra of low salt extract and RNA isolated from the extract of chick embryo brain chromatin. (—) Extract in 0.3 *M* salt; (- - - -) DEAE-isolated RNA in 10^{-2} *M* NaCl. No attempt has been made to normalize the curves relative to each other.

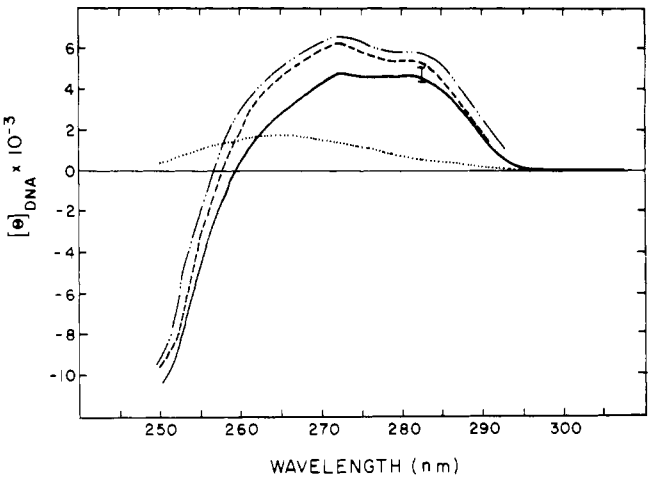

FIGURE 8: Circular dichroism of nucleoprotein low salt and mathematically constructed chromatin and native chromatin, from chick embryo brain (—); nucleoprotein low salt; (• • • •) low salt extract × 0.041; (- - - -) calculated chromatin CD; (— • • —) native chromatin. The error bar is plus and minus one standard deviation and applies approximately for the nucleoprotein low salt and both chromatin spectra.

DNA and proteins are to be assumed then the total contribution of DNA and/or protein to the CD between 255 and 275 nm of the low salt extracts relative to that of RNA must be the same in both the pig and chick samples. This requirement can be met if one assumes that in both the pig and chick low salt extracts the DNA and/or protein chromophores that contribute to the spectrum in this region are extracted to the same amount relative to RNA extracted. Therefore, it is attractive to speculate that the chromophores contributing to the CD of the low salt extracts are interacting in an ordered particle. This hypothesis makes any of the above requirements plausible: constant stoichiometry and unusual CD—by way of conformations resulting from the component interactions—could both be accounted for in this manner. If the extracted material contains such a particle of RNA plus DNA and/or protein it cannot be any of the ribonucleoprotein particles which are associated with either ribosomes (removed by saline-EDTA) or "informosomes" are all larger in molecular weight than the chromatin RNAs reported here (Samarina et al., 1968; Niessing and Sekeris 1970).

Reconstruction of the Circular Dichroism of Total Chromatin. It remains to be demonstrated that the CD of the low salt extract observed in solution also exists to the same extent in chromatin. We have demonstrated that the CD of the low salt extracts are either due entirely to RNA or are due to RNA plus a contribution from DNA and/or protein chromophores which are extracted in a constant ratio with the RNA. In either case the CD of the low salt extract need only be multiplied by the number of decimoles of chromosomal RNA extracted/dmol of DNA in the chromatin to reconstruct the contribution of the low salt extractable material to the CD of total chromatin. In Table III we show that for every μg of DNA in a chick embryo brain chromatin sample 0.043 μg of RNA is removed by low salt. This is equivalent to 0.041 dmol of RNA removed/dmol of DNA. Multiplying the CD of the chick embryo brain low salt extract by this factor, and adding the result to the spectrum of chick embryo brain low salt extracted chromatin, we obtain a spectrum that is indistinguishable from the CD of chick embryo brain chromatin (Figure 8). Thus the CD of the extractable RNA, DNA, and protein in chromatin is the same as that occurring in the low salt extract fraction. Further, this indicates that the ellipticity of the chromosomal DNA is the same before and after extraction. Therefore, no influence on the CD of the chromosomal DNA by low salt extractable RNA and/or DNA non-histone proteins is detectable.

Conclusion

The evidence shows that the presence of a 273-nm peak in the CD spectra of these samples correlates best with the total amount of RNA in the sample: removal of a salt-extractable fraction containing RNA along with salt soluble non-histone proteins and DNA causes attenuation of this peak. The salt-extractable material—possibly a particle—isolated from certain chromatins by solutions of low ionic strength (0.2–0.35) is present in the same form in chromatin as in the low salt extract. The low salt extract does not contain either of the ribonucleoprotein particles usually found with chromatin ribosomes, and informosomes—the size of the low salt extract associated RNA is much too small (4 S–11 S, see above). The presence of the loosely associated material in the various chromatins—as indicated by the presence of a 273-nm peak—may be closely associated with transcription activity of the chromatin. In this light it is interesting that chromatin extracted with low salt loses the capacity to synthesize RNA (Marzluff and Huang, 1974).

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