Circular Dichroism Studies of Histone–Deoxyribonucleic Acid Complexes. A Comparison of Complexes with Histone I (f-1), Histone IV (f2a1), and Their Mixtures*

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ABSTRACT: DNA complexes with histone I (f-1) and histone IV (f2a1) formed under identical conditions of salt gradient dialysis in the presence of 5 M urea exhibit different circular dichroism (CD) changes as measured in 0.14 M NaF. The difference CD spectra between histone complexes and DNA reveal some interesting features. Different CD spectra of histone IV–DNA complexes show a maximum at 260 nm, a shoulder at 245 nm, and another maximum at about 190 nm. Two minima were observed at 295 and 227 nm. Difference CD spectra of f-1–DNA complexes show two minima at 270 nm and at about 210 nm. However, the profiles of the peak values at 260 nm for histone IV–DNA complexes and at 270 nm for histone f-1–DNA complexes *vs.* histone:DNA ratio are both sigmoid rather than linear and occur at different ratios. This strongly indicates *cooperative* structural transitions are involved in the formation of specific aggregates, associated with conformational changes of the DNA. The DNA complexes formed with mixtures of histone f-1 and histone IV at various ratios show less CD change than with the individual histones. A small amount of f-1 is effectively able to block changes induced by IV, while IV blocks f-1 at relatively higher proportions. A mixture of these two histones, therefore, acts antagonistically in inducing CD changes. Complexes of sonicated DNA with these two histones show much larger CD changes. The implication of these findings on the structure of chromatin is discussed. CD spectra of these two histones in soluble complexes which show no CD changes of the DNA spectrum were obtained in 0.01 M NaF. The spectrum of bound f-1 is very similar to that of denatured proteins or random coil polypeptides. The CD spectrum of bound histone IV is close to that of proteins containing some α-helical structure (Shih, T. Y., and Fasman, G. D. (1971), *Biochemistry 10*, 1675). Therefore, it appears that f-1 bound to DNA is largely in the extended conformation, while histone IV may assume some α-helical conformation. These different conformations may partially be the reason for the different specific aggregation of DNA complexes with these two histones, which result in different CD changes of DNA.

Chromosomes of eukaryotic cells are complexes of DNA with basic proteins (the histones), acidic nonhistone proteins, and some RNA. Histones are the group of chromosomal proteins best characterized. In most cells, five major histone species are known, *i.e.*, I (f-1), IIb1 (f2a2), IIb2 (f2b), III (f3), and IV (f2a1) (Fambrough and Bonner, 1969; Stellwagen and Cole, 1969; Butler *et al.*, 1968; Georgiev, 1969; Hnilica, 1967).

Altered circular dichroism spectra of DNA in DNA complexes with histone f-1 and histone IV in 0.14 M salt have been reported (Fasman *et al.*, 1970b; Shih and Fasman, 1971; Olins and Olins, 1971). However, under certain other experimental conditions, *i.e.*, soluble complexes at low ionic strength, the CD[1] spectrum of DNA is unchanged (Olins, 1969; Wagner, 1970; Li *et al.*, 1971; Fasman *et al.*, 1970b; Shih and Fasman, 1971). It has been shown that the extent of the CD alteration of histone f-1–DNA complexes is affected by different salts and dioxane (Adler and Fasman, 1971), and by phosphorylation of histone f-1 (Adler *et al.*, 1971). It was further shown that the C-terminal fragment of f-1 histone which is rich in lysine residues induces greater CD change than does the intact f-1 (Fasman *et al.*, 1971). Alteration of the optical activity of polynucleotides or DNA by association with other basic proteins or polypeptides has also been demonstrated, *e.g.*, in poly-L-lysine–poly(A) (double-stranded) complexes (Davidson and Fasman, 1971), poly-L-lysine–DNA complexes (Cohen and Kidson, 1968; Shapiro *et al.*, 1969), and protamine (clupeine)–DNA complexes (Inoue and Ando, 1970). These changes of the CD spectra of DNA have been interpreted as an indication of a conformational change of the DNA (*e.g.*, Fasman *et al.*, 1970b; Shih and Fasman, 1971; Adler and Fasman, 1971).

Chromatin, the interphase chromosome, is a DNA complex with a mixture of histones and some other components (Bonner *et al.*, 1968). DNA in chromatin exhibits a CD spectrum different from that of partially dissociated or the isolated DNA (Shih and Fasman, 1970; Permogorov *et al.*, 1970; Simpson and Sober, 1970; Wilhelm *et al.*, 1970; Henson and Walker, 1970; Wagner and Spelsberg, 1971). Altered optical activity of DNA in chromatin has also been demonstrated from optical rotatory dispersion studies (Tuan and Bonner, 1969; Sponar *et al.*, 1970). As a step toward understanding the native complex, reassociated DNA complexes with mixtures of histone f-1 and histone IV have been studied. A more detailed comparative study of the CD of DNA complexes with these two histones is also reported.

Materials and Methods

Histone Preparations. Calf thymus histone IV (f2a1) was prepared as previously described (Shih and Fasman, 1971),

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[1] Abbreviations used are: CD, circular dichroism; SDS, sodium dodecyl sulfate.

as was histone f-1 (I) (Fasman *et al.*, 1970b). Both histones moved as a single band on polyacrylamide gel electrophoresis (Fambrough and Bonner, 1969).

DNA Preparations. Calf thymus DNA was prepared as before (Fasman *et al.*, 1970b). Sedimentation coefficients were determined by band sedimentation with a Beckman Model E ultracentrifuge (Vinograd *et al.*, 1963). Bulk solution used was 99% D_2O, 0.1 M NaCl, and 0.01 M Tris-HCl (pH 7.8). The median standard sedimentation coefficient, $s_{20,w}$ was 26 S for the isolated DNA which corresponds to a molecular weight of 13×10^6 (Studier, 1965). For studying the effect of DNA size, the DNA was broken down by ultrasonication with a Sonifier cell disruptor (Heat Systems Co., Melville, Long Island, N. Y.) for a total of 9 min at power level 5. DNA was dissolved in 0.14 M NaF–0.001 M Tris (pH 7.0) and immersed in ice water to prevent heat denaturation during sonication. The sonicated DNA had a median $s_{20,w}$ of 12 S which corresponds to a molecular weight of 1.5×10^6, and was much more homogeneous in size distribution than was untreated DNA.

Complex Preparations. Histone–DNA complexes were prepared by a salt gradient dialysis from 2 to 0.15 M NaCl in the presence of 5 M urea at 4°. Urea was removed by dialysis against this salt concentration. This procedure is termed dialysis A. To obtain CD spectra of the bound histone in complexes, gradient dialysis was extended to 0.015 M NaCl and urea then removed at this salt concentration. For the CD measurements the complexes were dialyzed in NaF solutions, because of the greater transparency of NaF. However, similar results are obtained in NaCl. The complexes thus prepared show no change in the CD of DNA. This procedure is called dialysis B (Shih and Fasman, 1971).

CD spectra were measured with a Cary 60 recording spectropolarimeter with a Model 6001 CD attachment. Spectra were obtained under nitrogen atmosphere at 23° in quartz cells of 1- or 0.5-cm path length. The concentration of samples was about 1 OD_{260}. The instrument was set for a slit program of 1.5 nm and the appropriate time constant and scanning speed were chosen (*e.g.*, time constant 3 and scanning speed 5). Mean residue ellipticity, $[\theta]$, is reported in (deg cm²)/dmole on the basis of DNA nucleotide concentration.

Absorption Spectra. Ultraviolet absorption spectra were measured with a Cary 14 recording spectrophotometer at 23°.

Concentration Determinations. The DNA concentration in the complexes was determined by uv absorption at 260 nm in the presence of 0.1% (w/v) SDS. The detergent dissociates the complex and clears up the turbidity. The absorption spectrum of DNA was unaffected in 0.1% SDS. Since histones show negligible absorption at 260 nm, this method has an accuracy of better than 1% for the determination of the DNA concentration. Therefore, precise ultraviolet absorption spectra of complexes could be obtained. Mean residue extinction coefficients, E_p, for pure DNA of 6800 was used for reduction of all spectra.

Stock solutions of histone IV were made up by weight, using samples dried at room temperature to constant weight over P_2O_5 in a vacuum desiccator at 0.01 mm. The mean residue weight of the HCl salt of histone IV is calculated to be 120 (DeLange *et al.*, 1969; Ogawa *et al.*, 1969). Concentrations of stock solutions of histone f-1 were determined by microbiuret reaction employing poly-L-lysine as standard (Zamenhof and Chargaff, 1957). Determination of histone IV concentration by the microbiuret agreed with weight determinations when a correction factor of 0.9 was used.

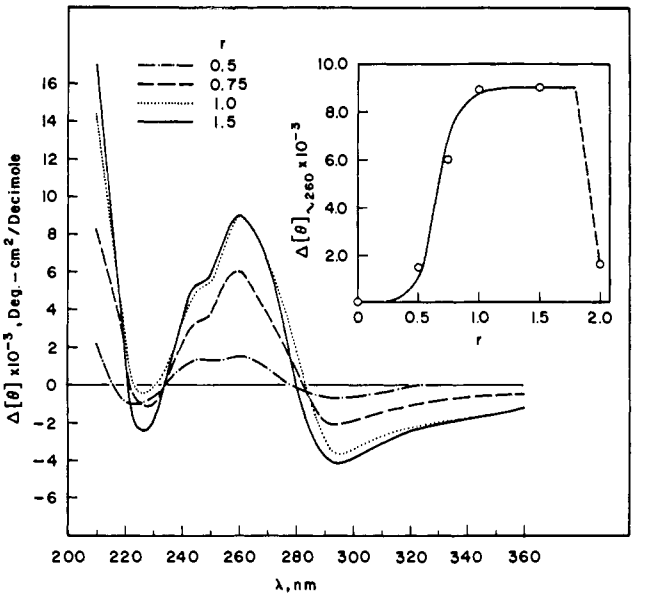

FIGURE 1: Difference CD spectra of histone IV–DNA complexes and DNA. Difference spectra $\Delta[\theta] = ([\theta]^{complex} - [\theta]^{DNA})$ were obtained by subtracting control DNA ($r = 0$) from complexes at different amino acid residue to nucleotide ratios (r), 0.5, —·—·—; 0.75, — — —; 1.0, ·····; 1.5, ————. The inset is $\Delta[\theta]$ at the 260-nm peak of the difference spectra as a function of r. DNA concentrations were 1.4×10^{-4} M (phosphate) and the path length of the cell used was 1 cm. Solvent was 0.14 M NaF–1 mM Tris (pH 7.0).

Results

A Comparative Circular Dichroism Study of DNA Complexes with Histone I (f-1) and Histone IV (f2a1). Histone f-1 induces a decrease and red shift in the positive 275-nm CD band of DNA and an increase in the magnitude of the 245-nm negative band (Fasman *et al.*, 1970b). The changes induced by histone IV are in the opposite direction, *i.e.*, the 275-nm positive band is enhanced and blue shifted, and the negative 245-nm band is decreased (Shih and Fasman, 1971). These changes were observed in 0.14 M NaF at neutral pH. However, conditions of complex formation with these two histones were not exactly the same. Changes in the f-1–DNA complexes were observed when complexes were prepared by a salt gradient dialysis. The histone IV–DNA complexes prepared by the same procedure failed to show any CD change. However, if complexes were made by salt gradient dialysis in the presence of 5 M urea, changes in the CD of histone IV–DNA complexes were observed. Presumably, the much greater tendency of histone IV than of histone f-1 to aggregate at high ionic strength, prevents proper complex formation. The effect of urea on the f-1–DNA complexes was not reported. In the present paper, data are presented which show that histone f-1–DNA complexes prepared in the presence of urea cause similar changes in the CD of DNA as those caused by salt-gradient dialysis in the absence of urea (Figure 5a). Therefore, it appears that the different types of changes in the CD spectra of DNA are due solely to different histones. The magnitude of the f-1–DNA CD changes, especially for complexes of larger ratios (amino acid residue:DNA phosphate), are larger for complexes formed in the presence of urea than in its absence; however, at ratios of 0.5 and lower the CD changes were the same. Apparently, diminished aggregation of DNA–histone complexes in the presence of urea during primary binding of histone and DNA facilitates the formation of characteristic complexes.

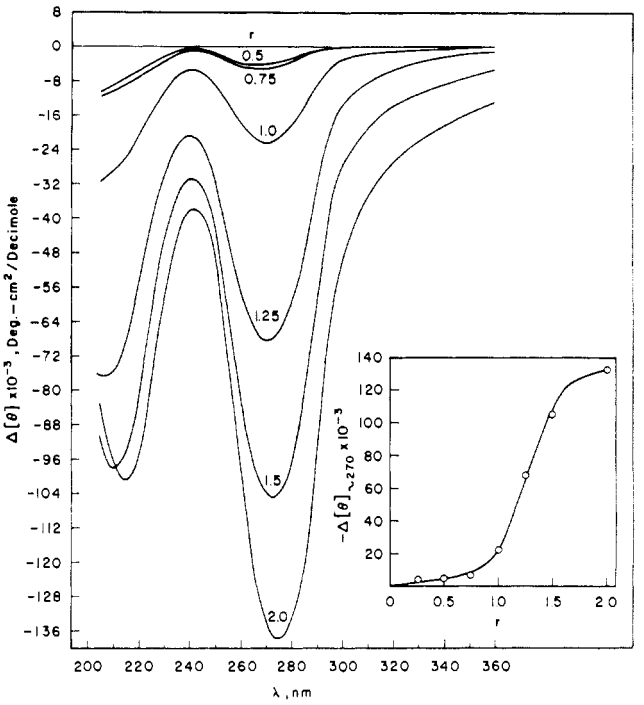

FIGURE 2: Difference CD spectra of histone f-1–DNA complexes and DNA. Difference spectra $\Delta[\theta] = ([\theta]^{complex} - [\theta]^{DNA})$ were obtained by subtracting control DNA from complexes at r values of 0.5, 0.75, 1.0, 1.25, 1.5, and 2.0. The inset is $-\Delta[\theta]$ at the 270-nm negative ellipticity band of the difference spectra as a function of r. DNA concentrations were 1.4×10^{-4} M (phosphate) and optical path length was 1 cm. Solvent was 0.14 M NaF–1 mM Tris (pH 7.0).

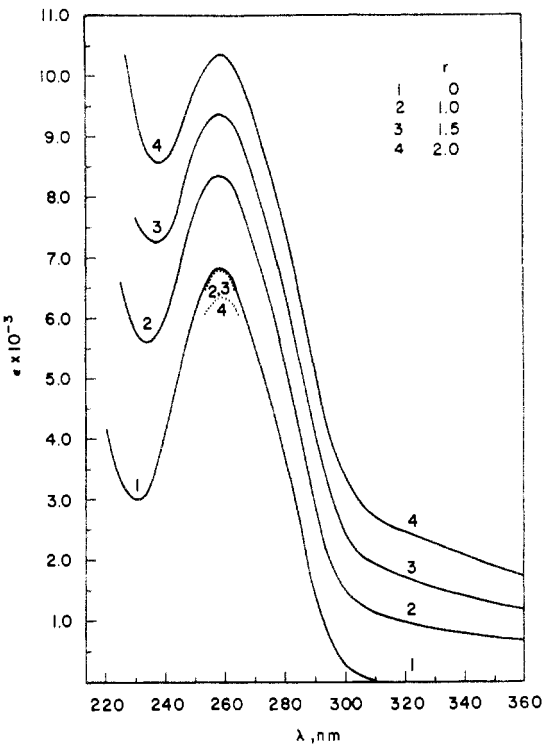

FIGURE 3: Ultraviolet absorption spectra of histone IV–DNA complexes. Spectra were measured in 0.14 M NaF–1 mM Tris (pH 7.0) at concentration of 1.3×10^{-4} M DNA (phosphate). Accurate DNA concentrations were determined by dissociating the complexes in 0.1% SDS and concentration was calculated using $E_p = 6800$. The dotted lines are peak values corrected for light scattering by extrapolation from 360 to 320 nm according to the equation, $\log OD = -a \log \lambda + C$.

Close examination of the changes in the CD spectra of DNA induced by complexing with histones, reveals some interesting features. In Figures 1 and 2 are seen the difference spectra obtained by subtracting the spectrum of the control DNA ($r = 0$) from those of complexes. In Figure 1 is seen the difference spectra of histone IV–DNA complexes at various histone:DNA ratios (r expressed in amino acid residue: nucleotide residue). The difference spectra have a maximum at 260 nm and a shoulder at 245 nm. The shoulder is more pronounced at low ratios. There is another maximum at about 190 nm. Two minima are observed at 295 and 227 nm. The difference spectra of the complexes, therefore, appear to have fine structure in the ultraviolet absorption region of the DNA bases. Figure 2 shows the difference spectra of histone f-1–DNA complexes. The difference spectra possess minima at 270 nm and at about 210 nm. These spectra are completely different from those of the histone IV–DNA complexes, and the amplitude of the changes is about one order of magnitude larger in the histone f-1–DNA complexes than with histone IV–DNA complexes.

The magnitude of the maximum at 260 nm for histone IV–DNA complexes and the minimum at 270 nm for histone f-1–DNA complexes varies as a function of r as shown in insets of Figures 1 and 2. Of particular significance is that the profiles are of sigmoid type rather than linear. This strongly indicates that there are *cooperative* structural transitions in DNA–histone complexes as a function of ratio. The r value at the inflection point for histone f-1–DNA complexes is about twice that for histone IV–DNA complexes, suggesting that the binding is stronger for IV than for f-1. This is consistent with observations of selective histone removal by salt and of histone–DNA reassociation studies (Ohlenbusch *et al.*, 1967; Akinrimisi *et al.*, 1965; Shih and Bonner, 1970a). The transition profile for histone IV–DNA complexes appears to be discontinuous at r larger than 1.5. The reason is not apparent.

The general features of the CD changes induced by these two histones are quite reproducible. However, the absolute values of the mean residue ellipticity, $[\theta]$, of histone–DNA complexes varied somewhat among different sets of experiments. Figures 1 and 2 are results of typical sets of experiments, while Figure 5a,b shows the average spectra of several experiments with their deviation. The formation of specific structures with characteristic CD changes appears to be a very delicate process. A great number of factors affect this structure formation, such as histone:DNA ratio, purity of histones, ionic strength (Fasman *et al.*, 1970b), urea (Shih and Fasman, 1971), specific salts (Adler and Fasman, 1971), phosphorylation of histone f-1 (Adler *et al.*, 1971), integrity of the intact histone (Fasman *et al.*, 1971), and some factors still not clearly understood.

Ultraviolet Absorption Spectra of Histone–DNA Complexes. In the present study, very accurate absorption spectra of the suspensions of histone–DNA particles were obtained, and compared to the absorption spectra of clear solutions of dissociated complexes in the presence of 0.1% SDS. SDS itself does not affect the absorption of DNA. Additional valuable information concerning the optical activity of histone–DNA particles can be extracted from detailed analysis of these spectra. As seen in Figure 3 for IV–DNA complexes, there is considerable scattering of light above 320 nm in which region DNA has no absorption. The scattering in the region between 320 and 360 nm is linear on a log OD *vs.* log λ plot. The extrap-

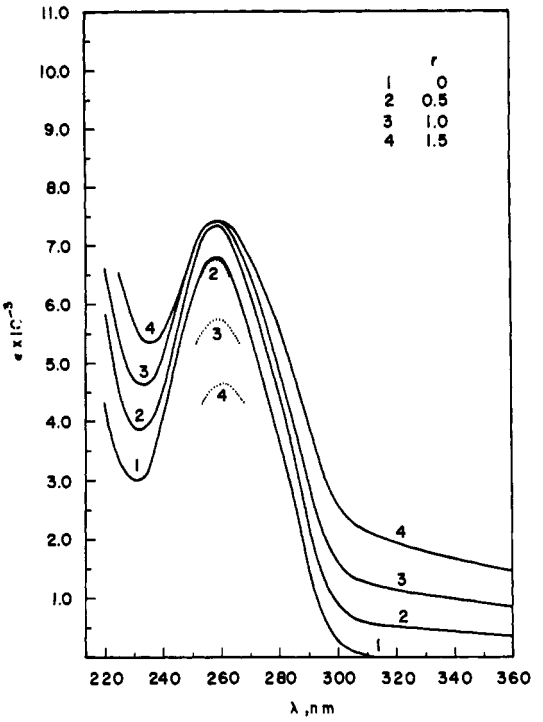

FIGURE 4: Ultraviolet absorption spectra of histone f-1–DNA complexes. Spectra were measured in 0.14 M NaF–1 mM Tris (pH 7.0) at concentration of 1.3×10^{-4} M DNA (phosphate). Accurate DNA concentrations were determined by dissociating the complexes in 0.1% SDS, and concentrations calculated using $E_p = 6800$. The dotted lines are peak values corrected for light scattering by extrapolation from 360 to 320 nm according to the equation, $\log OD = -a \log \lambda + C$.

olated contribution of Rayleigh scattering at the 257.5-nm absorption maximum can be obtained. Except at very high ratios (curve 4, $r = 2.0$), the corrected values of the complex absorption agree very closely with the absorption of the dissociated DNA. Therefore, the absorption flattening effect due to shadowing of chromophores in the particle (Duysens, 1956) in this system appears to be very small. Absorption spectra of histone f-1–DNA complexes are seen in Figure 4. There is a considerable hypochromic effect remaining in the corrected spectra of complexes in which Rayleigh scattering has been subtracted by extrapolation from 320- through 360-nm region. Here two possibilities exist. (1) The apparent hypochromic effect is due to considerable absorption flattening because of the large particle sizes. (2) The hypochromic effect represents true absorption changes as might be anticipated to be associated with the huge CD changes (Figure 2). If the absorption flattening effect is significant, the observed CD should underestimate the true value, and therefore, the true CD changes would be much greater than those observed here.

DNA Complexes with Mixtures of Histone F-1 and Histone IV. Chromatin of eukaryotic cells is a DNA complex with five major histone species. It is, therefore, of interest to examine reconstituted complexes formed with mixtures of different histones in order to evaluate their relative contributions to the final CD spectrum. Histone f-1 and histone IV induce different types of CD changes (Figure 5a,b). Therefore it is of interest to enquire whether these two histones act additively, or if not, synergistically or antagonistically in altering the CD spectrum of DNA. In Figure 5c is seen the CD of complexes with histones f-1 and IV mixed on a 1:1 amino acid residue basis. There is evidence of the CD spectral changes typical of

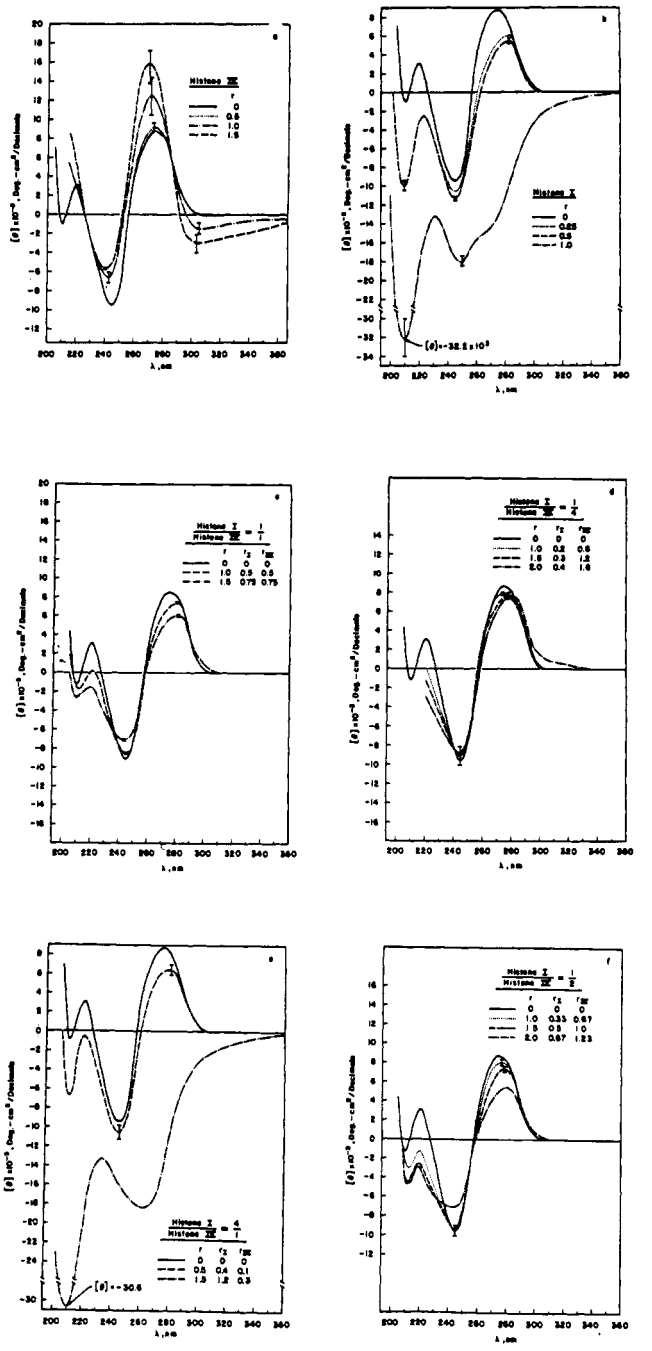

FIGURE 5: Circular dichroism spectrum of DNA complexes with mixtures of histone f-1 and histone IV at various ratios. (a) Histone IV alone. Histone:DNA ratios were ———, 0; · · · · ·, 0.5; —·—·—, 1.0 and — — —, 1.5. (b) Histone f-1 alone. Histone:DNA ratios were ———, 0; · · · · ·, 0.25; — — —, 0.5; and —·—·—, 1.0. (c) Mixture with f-1 to IV ratio of 1. Histone:DNA ratios were ———, 0; — — —, 1.0 and —·—·—, 1.5. (d) Mixture with f-1 to IV ratio of 1 to 4. Histone:DNA ratios were ———, 0; · · · · ·, 1.0, — — —, 1.5 and —·—·—, 2.0. (e) Mixture with f-1 to IV ratio of 4 to 1. Histone:DNA ratios were ———, 0; — — —, 0.5; and —·—·—, 1.5. (f) Mixture with f-1 to IV ratio of 1 to 2. Histone:DNA ratios were ———, 0; · · · · ·, 1.0; — — —, 1.5 and —·—·—, 2.0. DNA concentration was 1.3×10^{-4} M phosphate and optical path length was 1 cm. Solvent was 0.14 M NaF–1 mM Tris (pH 7.0). Error bars show values of two independent experiments.

histone f-1 as indicated by the 275-nm positive CD band, although the changes are smaller in magnitude by comparison to the spectral change induced by the same amount of f-1

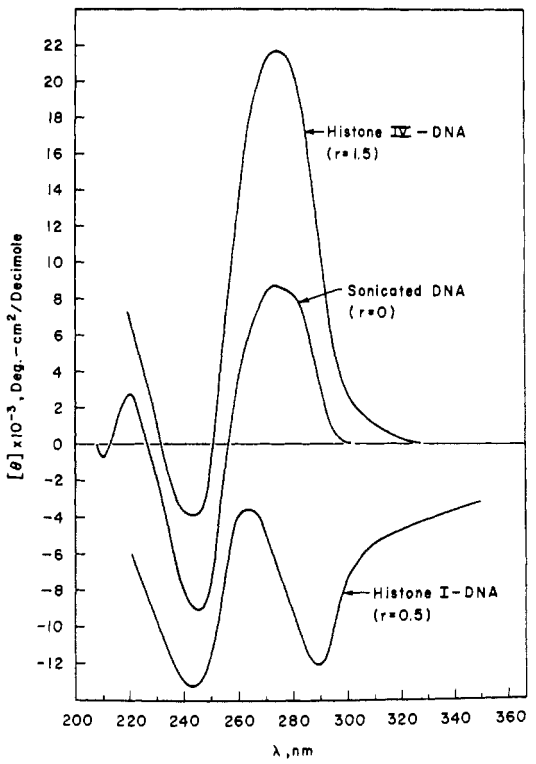

FIGURE 6: Histone complexes with sonicated DNA. Histone:DNA ratio for histone f-1–DNA complex was 0.5 and for histone IV–DNA complex, 1.5. DNA concentration was 1.3×10^{-4} M phosphate and optical path length, 1 cm. Solvent was 0.15 M NaCl–0.01 M Tris (pH 7.0).

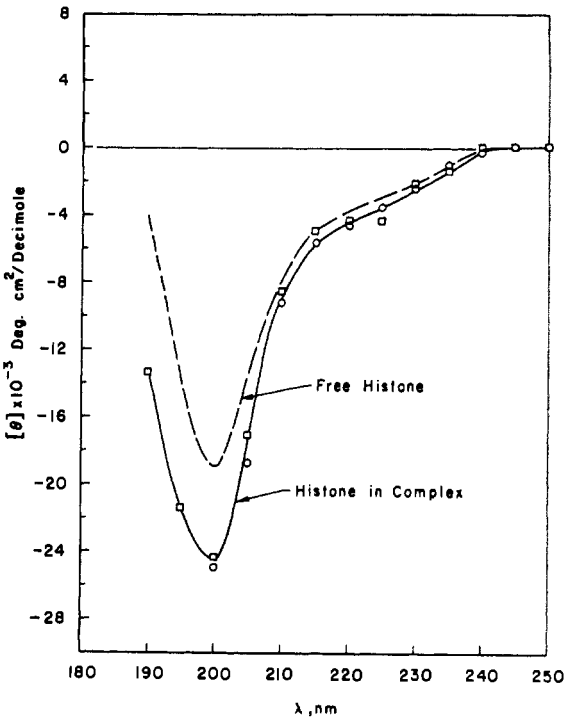

FIGURE 7: Circular dichroism spectra of histone f-1 bound to the unchanged B conformation of DNA. Histone f-1–DNA complexes were prepared by dialysis B and show no change in DNA CD spectra. CD of histone in complex of $r = 1.0$ (solid line) was obtained by subtracting CD of control DNA ($r = 0$) from that of complex measured at a similar concentration of 1.12×10^{-4} M. Optical path lengths of 0.5 (□) and 1.0 cm (○) were used. $[\theta]$ was based on amino acid residue concentration of histone f-1. Solvent was 0.01 M NaF. Dashed line is the spectra of free histone f-1 in 0.14 M NaF which is similar to that in 0.01 M NaF.

alone (Figure 5b). However, there is no trace of the typical spectral change due to histone IV. In Figure 5d is seen the CD of complexes with a histone f-1 to histone IV ratio of 1:4. Very little change of the DNA CD spectrum was observed. Therefore, it seems that a small amount of histone f-1 is effectively able to block histone IV. On the other hand, a small amount of IV (f-1:IV = 4:1) shows little effect on f-1 (Figure 5e). A significant effect shows up only at relatively higher proportion of IV (f-1:IV = 1:2, Figure 5f).

From the above results it is concluded that these two histones do not act additively in inducing CD changes of the DNA. Histone f-1 appears antagonistic to and effectively blocks histone IV at low relative amounts while histone IV blocks f-1 less effectively and requires far greater amounts.

Effect of Size of DNA Molecules on the CD Changes of its Histone Complexes. The exact length of DNA is eukaryotic chromosomes is still a mystery. Purified DNA from higher organisms is the breakdown product of heterogeneous sizes. A study of the size-effect is pertinent to an understanding of the native chromatin complex. In Figure 6 is seen the CD of complexes formed with DNA sonicated to a molecular weight of 1.5×10^6. The CD spectrum of sonicated DNA is not changed as compared to the DNA stock solution with a median molecular weight of 13×10^6. However, the complexes with histone f-1 and histone IV exhibit very enhanced CD changes. Histone f-1–DNA complex at $r = 0.5$ shows a CD spectrum close to that of complexes with unsonicated DNA at much higher ratios. The changes induced by histone IV are also larger than that with the original DNA. It is quite clear that smaller and more homogeneous fragments of the sonicated DNA react more efficiently with histones to form particles with enhanced characteristic CD spectra. The cooperative effects of binding, discussed above, are more effective when the DNA is of shorter length, as might be expected. The effect of DNA size is also apparent in protamine–DNA complexes; protamine complexes with sonicated DNA causes larger changes in optical activity of DNA (Inone and Ando, 1970) than with larger DNA (D. Carroll, 1971, private communication.

Conformation of Histone f-1 Bound to DNA in the B Conformation. Histone–DNA complexes prepared by Dialysis B show no change in DNA CD spectrum. Therefore, the CD of the bound histone in this type of complex can be obtained by subtracting the DNA CD spectrum from that of the complex. In Figure 7 is seen the CD spectrum of bound histone f-1 which is very similar to the CD of free histone, and is essentially a random coil. The small increase in $[\theta]_{200}$ might indicate a more extended conformation when bound, similar to that found for poly-α-amino acids (Fasman *et al.*, 1970a). This is in strong contrast to the CD of bound histone IV previously published (Shih and Fasman, 1971), which shows a negative band at 220 nm and a positive band at about 190 nm. This spectrum is close to that of proteins containing some α-helical structure. It is quite clear that the conformation assumed by these two histones when bound to DNA is quite different. It is also interesting to note that the CD of free histone IV in low ionic strength (0.01 M) is essentially a random coil; on increasing the ionic strength to 0.14 M the CD spectrum becomes similar to that of proteins containing considerable α-helical structure (Shih and Fasman, 1971). The same conclusion has been reported utilizing optical rotatory dispersion

(Bradbury *et al.*, 1965; Jirgensons and Hnilica, 1965). The CD spectrum of histone f-1, however, does not show this kind of salt dependence.

Discussion

The CD spectrum of DNA in chromatin is different from that of isolated DNA, which suggests a conformation alteration; the changes are completely reversed by dissociation of most of the chromosomal proteins with sodium dodecyl sulfate (Shih and Fasman, 1970). Many other studies utilizing optical rotatory dispersion or circular dichroism agree with this conclusion and further suggest that the lysine-rich histone I (f-1) is not responsible for the change of the optical activity of DNA, since removal of this histone species by NaCl does not affect the altered spectrum of DNA (Tuan and Bonner, 1969; Sponar *et al.*, 1970; Permogorov *et al.*, 1970; Simpson and Sober, 1970; Wilhelm *et al.*, 1970; Henson and Walker, 1970). Wagner and Spelsberg (1971), however, observed that the CD of chromatin is not restored to that of DNA until removal of histone IV. The conclusions from the selective histone removal studies, concerning the role of individual histones in causing the CD changes of DNA, are not consistent and are in apparent disagreement with the results obtained from reassociated DNA complexes with purified histones. The reduced magnitude of the 275-nm positive CD band of DNA in chromatin is very similar to the type of CD change induced by histone f-1 at small r ratios. In chromatin there is no trace of the type of change characteristic of histone IV–DNA complexes. The results of DNA complexes with mixtures of histone f-1 and IV show that a small amount of histone f-1 is effectively able to block the change induced by histone IV. Therefore, in chromatin no trace of the effect of histone IV would be anticipated. Another observation pertinent to the smaller change in chromatin is the size effect of DNA molecules on the CD of histone–DNA complexes. In cells of higher organisms, DNA is present as strands as long as several millimeters (Huberman and Riggs, 1966). The isolated DNA is the breakdown product of much smaller size (median molecular weight 13×10^6). The histone complexes with DNA further broken down by ultrasonication (mol wt 1.5×10^6) demonstrate much enhanced CD changes. It is anticipated that histone complexes with much larger molecular weight DNA in the native state will show even less CD change, in accord with the CD of chromatin. It is, therefore, postulated that histone f-1 is one of the major histone species responsible for the CD change found in chromatin. The apparent contradiction with selective histone removal studies can perhaps be explained as follows: histone f-1 may be directly responsible for the structure with an altered CD spectrum; but once the structure is formed, the other histones hold this conformation intact; removal of small amount of proteins, *e.g.*, histone f-1 and maybe other histones, will not disrupt this preformed structure, and the CD of the residual complexes remains the same. However, other factors may also be operative, *e.g.*, specific histone–histone interaction, specific partial binding of histone molecules, due to their conformation, specific order of binding due to different binding strength, and specificity of the histone–DNA interaction due to DNA base sequences.

On the question of the origin of the CD spectral changes in DNA complexes, the present study provides some interesting observations. The difference CD spectra of DNA complexes minus DNA (Figures 1 and 2) show very clearly that the spectral alterations are most pronounced in the absorption region of the DNA purine and pyrimidine bases, and that there is CD spectral fine structure in the histone IV–DNA complexes. Therefore, any speculation on the origin of the spectral change must take into account this observation, and the simple liquid crystal explanation of the observed CD changes (Haynes *et al.*, 1970) seems inadequate (Adler and Fasman, 1971). In spite of many X-ray studies, it is still not known definitively whether the B form of DNA is retained in unaltered form in nucleohistone (Garrett, 1971; Bram, 1971; Haynes *et al.*, 1970). The magnitude of the CD changes, $\Delta[\theta]$, *vs.* histone:DNA ratio possesses sigmoid rather than linear profiles, which strongly indicates that some type of cooperative structural transitions is involved, associated with the conformational changes of DNA.

Two major artifacts of the optical activity of suspensions are recognized (Ottaway and Wetlaufer, 1970; Urry *et al.*, 1970; Gordon and Holzwarth, 1971; Schneider *et al.*, 1970). First, scattering of the light beam by particles might distort the CD spectrum. Since CD is the measurement of the difference in absorption between left and right circularly polarized light, only asymmetric but not symmetric scattering of these two polarized components contribute to the measured CD. The direct experimental measurement of the magnitude of asymmetric scattering in the present systems is still unavailable. But a theoretical consideration concludes that for particles of polypeptides or polynucleotides with radius less than 1μ, the scattering distortion of CD spectrum is quite small (Ottaway and Wetlaufer, 1970). The second possible artifact is the flattening effect of the absorption and optical activity spectra near the absorption region, arising from the shadowing effect of chromophores in the particles, which is equivalent to the reduction of the effective concentration (Duysens, 1956). From the analysis of absorption spectra of histone IV–DNA complexes (Figure 3), it appears that the flattening effect, if there is any, is very small. However, for histone f-1–DNA complexes at $r > 0.5$, this artifact appears to be more pronounced (Figure 4), although it is quite possible that the hypochromism is a true absorption change and is directly associated with huge CD changes. If there is indeed significant flattening effect, the observed $[\theta]$ should underestimate its true value, and true CD changes should be greater than reported here. Other pertinent experimental observations which rule out the possibility that the observed CD changes are due entirely to artifacts have been discussed in previous publications (Fasman *et al.*, 1970b; Shih and Fasman, 1971).

In conclusion, a molecular picture most consistent with the CD observations can be conceived. Previously it has been demonstrated that cooperative primary binding of histones and DNA occurs at some point during the salt gradient dialysis; likewise has the binding of poly-L-lysine to DNA (Akinrimisi *et al.*, 1965; Tsuboi *et al.*, 1966; Olins, 1969; Shapiro *et al.*, 1969; Shih and Bonner, 1970a,b). Under reversible conditions, a most stable structure is formed which is determined by DNA base lattices and histone amino acid sequences. This structure is consolidated upon further lowering the ionic strength, in which the binding is relatively irreversible. Complexes with characteristic T_m have also been demonstrated by heating the mixture of histone and DNA in 3.6 M urea, 5 mM sodium cacodylate, 0.14 mM sodium Versenate, and 0.011 M standard saline citrate at pH 7.0 (Ansevin and Brown, 1971). Upon removal of urea at 0.15 M NaCl, in which the nucleohistone has the least solubility, the complexes associate concomitantly with strengthened hydrophobic and hydrogen bonding to form specific aggregates with characteristic CD changes. This second process is also cooperative

with respect to histone:DNA ratios. Aggregation-induced conformational changes of the geometry of DNA bases or interaction of chromophores between helices might occur, as has been suggested for polypeptide–polypeptide interaction (Hammes and Schullery, 1968). This kind of specific aggregation may also be conceived of as the formation of the supercoil structure suggested for nucleohistone (Pardon *et al.*, 1967). The difference in the CD changes induced in DNA by histone IV and by histone f-1, which is related to different structural changes, can be accounted for by the difference in amino acid sequence and composition, and their conformation when bound to DNA. The CD spectrum of histone IV in complexes in which the DNA conformation is not changed is close to that of proteins containing some α-helical conformation (Shih and Fasman, 1971). A very similar CD spectrum has also been observed by Li *et al.* (1971). However, histone f-1 bound to DNA is largely in an extended conformation and the CD spectrum is very similar to that of a denatured protein (Figure 7) (Fasman *et al*, 1970b). The unusually high proline content of f-1 may be one of the reasons for this extended conformation. It is interesting to note that the huge CD changes of DNA complexes with poly-L-lysine, which may be also in the extended conformation, are very similar to that of complexes with f-1 (Shapiro *et al.*, 1969; D. Carroll, 1971, private communication). These two histones appear to counteract each other in the formation of the specific aggregates, with f-1 being especially effective.

It is quite tempting to relate the specific aggregation suggested by CD to the characteristic morphological changes of chromosomes during cell division. The CD spectrum of isolated metaphase chromosomes has been reported, and does not show the CD changes observed here (Cantor and Hearst, 1970). However, the compositional complexity and possible spectral distortion due to large particle size may obscure the CD changes.

References

Adler, A. J., and Fasman, G. D. (1971), *J. Phys. Chem. 75*, 1516.

Adler, A. J., Schaffhausen, B., Langan, T. A., and Fasman, G. D. (1971), *Biochemistry 10*, 909.

Akinrimisi, E. O., Bonner, J., and Ts'o, P. O. P. (1965), *J. Mol. Biol. 11*, 128.

Ansevin, A. T., and Brown, B. W. (1971), *Biochemistry 10*, 1133.

Bonner, J.; Dahmus, M. E., Fambrough, D., Huang, R. C. C., Marushige, K., and Tuan, D. Y. H. (1968), *Science 159*, 47.

Bradbury, E. M., Crane-Robinson, C., Johns, E. W., Phillips, D. M. P., and Murray, K. (1965), *Nature (London) 205*, 1315.

Bram, S. (1971), *J. Mol. Biol. 58*, 277.

Butler, J. A. V., Johns, E. W., and Phillips, D. M. P. (1968), *Progr. Biophys. Mol. Biol. 18*, 209.

Cantor, K. P., and Hearst, J. E. (1970), *J. Mol. Biol. 49*, 213.

Cohen, P., and Kidson, C. (1968), *J. Mol. Biol. 35*, 241.

Davidson, B., and Fasman, G. D. (1971), *Arch. Biochem. Biophys. 144*, 650.

DeLange, R. J., Fambrough, D. M., Smith, E. L., and Bonner, J. (1969), *J. Biol. Chem. 244*, 319.

Duysens, L. N. M. (1956), *Biochim. Biophys. Acta 19*, 1.

Fambrough, D. M., and Bonner, J. (1969), *Biochim. Biophys. Acta 175*, 113.

Fasman, G. D., Hoving, H., and Timasheff, S. N. (1970a), *Biochemistry 9*, 3316.

Fasman, G. D., Schaffhausen, B., Goldsmith, L., and Adler, A. J. (1970b), *Biochemistry 9*, 2814.

Fasman, G. D., Valenzuela, M. S., and Adler, A. J. (1971), *Biochemistry 10*, 3795.

Garrett, R. A. (1971), *Biochemistry 10*, 2227.

Georgiev, G. P. (1969), *Annu. Rev. Genet. 3*, 155.

Gordon, D. J., and Holzwarth, G. (1971), *Arch. Biochem. Biophys. 142*, 481.

Hammes, G. G., and Schullery, S. E. (1968), *Biochemistry 7*, 3882.

Haynes, M., Garrett, R. A., and Gratzer, W. B. (1970), *Biochemistry 9*, 4410.

Henson, P., and Walker, I. O. (1970), *Eur. J. Biochem. 16*, 524.

Hnilica, L. S. (1967), *Progr. Nucl. Acid Res. Mol. Biol. 7*, 25.

Huberman, J. A., and Riggs, A. D. (1966), *Proc. Nat. Acad. Sci. U. S. 55*, 599.

Inoue, S., and Ando, T. (1970), *Biochemistry 9*, 395.

Jirgensons, B., and Hnilica, L. S. (1965), *Biochim. Biophys. Acta 109*, 241.

Li, H. J., Isenberg, I., and Johnson, Jr., W. C. (1971), *Biochemistry 10*, 2587.

Ogawa, Y., Quagliarotti, G., Jordan, J., Taylor, C. W., Starbuck, W. C., and Busch, H. (1969), *J. Biol. Chem. 244*, 4387.

Ohlenbusch, H. H., Olivera, B. M., Tuan, D., and Davidson, N. (1967), *J. Mol. Biol. 25*, 299.

Olins, D. E. (1969), *J. Mol. Biol. 43*, 439.

Olins, D. E., and Olins, A. L. (1971), *J. Mol. Biol. 57*, 437.

Ottaway, C. A., and Wetlaufer, D. B. (1970), *Arch. Biochem. Biophys. 139*, 257.

Pardon, J. F., Wilkins, M. H. F., and Richards, B. M. (1967), *Nature (London) 215*, 508.

Permogorov, V. I., Debabov, V. G., Sladkova, I. A., and Rebentish, B. A. (1970), *Biochim. Biophys. Acta 199*, 556.

Schneider, A. S., Schneider, M. T., and Rosenheck, K. (1970), *Proc. Nat. Acad. Sci. U. S. 66*, 793.

Shapiro, J. T., Leng, M., and Felsenfeld, G. (1969), *Biochemistry 8*, 3219.

Shih, T. Y., and Bonner, J. (1970a), *J. Mol. Biol. 48*, 469.

Shih, T. Y., and Bonner, J. (1970b), *J. Mol. Biol. 50*, 333.

Shih, T. Y., and Fasman, G. D. (1970), *J. Mol. Biol. 52*, 125.

Shih, T. Y., and Fasman, G. D. (1971), *Biochemistry 10*, 1675.

Simpson, R. T., and Sober, H. A. (1970), *Biochemistry 9*, 3103.

Sponar, J., Boublik, M., Fric, I., and Sormova, Z. (1970), *Biochim. Biophys. Acta 209*, 532.

Stellwagen, R. H., and Cole, R. D. (1969), *Annu. Rev. Biochem. 38*, 951.

Studier, F. W. (1965), *J. Mol. Biol. 11*, 373.

Tsuboi, M., Matsuo, K., and Tso, P. O. P. (1966), *J. Mol. Biol. 15*, 256.

Tuan, D. Y. H., and Bonner, J. (1969), *J. Mol. Biol. 45*, 59.

Urry, D. W., Hinners, T. A., and Masotti, L. (1970), *Arch. Biochem. Biophys. 137*, 214.

Vinograd, J., Bruner, R., Kent, R., and Weigle, H. (1963), *Proc. Nat. Acad. Sci. U. S. 49*, 902.

Wagner, T. E. (1970), *Nature (London) 227*, 65.

Wagner, T., and Spelsberg, T. C. (1971), *Biochemistry 10*, 2599.

Wilhelm, X., Champagne, M., and Daune, M. (1970), *Eur. J. Biochem. 15*, 321.

Zamenhof, S., and Chargaff, E. (1957), *Methods Enzymol. 3*, 702.