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Complexes of Deoxyribonucleic Acid with Fragments of Lysine-Rich Histone (f-1). Circular Dichroism Studies*

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ABSTRACT: Lysine-rich (f-1) histone from calf thymus was cleaved by *N*-bromosuccinimide, to yield a large carboxyl-terminal polypeptide of high lysine content and a smaller amino-terminal fragment with relatively low lysine content. Reconstituted complexes of calf thymus DNA with these two fragments were examined by means of circular dichroism. Although the N-terminal fragment (6000 molecular weight) failed to cause significant change in DNA circular dichroism upon complexation, the C-terminal fragment (15,000 molecular weight) effected even larger changes than did equivalent molar amounts of intact f-1. These observations were not simply related to differences in lysine content of the fragments, or to the reduced binding affinity of the N-terminal fragment for DNA. The two f-1 fragments showed no tendency to interact with each other in the absence of DNA. However, complexes formed with DNA and a mixture of the two polypeptides (in such amounts as to reconstitute f-1) exhibited augmented circular dichroism changes. This finding suggests that, although binding of the highly cationic C-terminal end of f-1 histone is the primary cause of conformational change in DNA, the N-terminal fraction of the molecule may modify this interaction. In the intact f-1 molecule, the N segment appears to moderate the conformational effect of the C segment upon DNA, while when cleaved from the C fragment it enhances this effect. This finding might suggest that in the intact f-1 molecule the N-terminal segment folds back on the C-terminal segment, thus acting as a modifier.

In the cell nucleus of higher organisms DNA is found complexed to basic proteins, the histones, as well as to other constituents. The interaction between histones and DNA is dominated by electrostatic forces; the histones in chromatin contain sufficient basic amino acid residues to neutralize nearly all the negative charges on the ionized phosphates of the DNA. However if any specificity between DNA and histones exists, other interactions must play a controlling role.

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Functions which have been proposed for histones are stabilization of the condensed structure of nuclear DNA, and regulation of transcription; the evidence has been reviewed by Elgin *et al.* (1971). The very lysine-rich histones (f-1) constitute about 20% of the total histone of a cell. The f-1 fraction exhibits species and tissue specificity (Bustin and Cole, 1968; Panyim and Chalkley, 1969) and limited heterogeneity (Kinkade and Cole, 1966; Bustin and Cole, 1969a), can be specifically phosphorylated *in vivo* (Langan, 1969), and in some tissues is also found outside the nucleus (Gurley *et al.*, 1970). The f-1 histone has a molecular weight of 21,000, and contains 29% lysine residues, little arginine, and a large amount of alanine and proline (Bustin and Cole, 1969b).

This laboratory has been studying the interaction of calf thymus DNA with f-1 histone from the same source (Fasman *et al.*, 1970; Adler *et al.*, 1971; Adler and Fasman, 1971). Circular dichroism studies of reassociated f-1–DNA complexes have shown that f-1 causes large changes in the conformation of DNA. The distortions of the DNA circular dichroism spectrum increase with the amount of histone in the complex (Fasman *et al.*, 1970), decrease if f-1 is phosphorylated at the serine-37 position (Adler *et al.*, 1971), and are dependent on the solvent and electrolyte present (Adler and Fasman, 1971). References to circular dichroic and other physical studies of chromatin and model systems can be found in the previous paper of this series (Adler and Fasman, 1971). The present study is concerned with complexes formed between DNA and two large polypeptides derived from f-1. The aim was to investigate whether any specificity in DNA binding can be demonstrated for these fragments, or whether any possible interactions between the various sections of the native histone molecule can be found.

Bustin and Cole (1969b) have succeeded in cleaving the f-1 molecule (mol wt 21,000, 29% lysine) by the action of *N*-bromosuccinimide on the only tyrosyl residue present. The product is two large fragments: the amino-terminal third of the protein (mol wt 6000, 63 peptide residues, 19% lysine) and the carboxyl-terminal remainder of the chain (mol wt 15,000, 152 residues, 33% lysine). (The lysine compositions are those given for unfractionated calf thymus f-1.) The N-terminal fragment is designated N in the present work (N_2 in Bustin and Cole's (1969b) terminology); the C-terminal polypeptide will be called C (fragment N_1 of Bustin and Cole). Examination of chymotryptic digests of a homogeneous f-1 subfraction (Bustin and Cole, 1970) shows that the distribution of amino acid residues in f-1 is far from uniform: 45 of the 59 lysine residues are clustered in the C-terminal half of the molecule, as are 16 of the 22 prolines. This distribution suggests that the carboxyl terminus may be the primary site of electrostatic binding of f-1 to DNA. The high cationic charge density, plus the lack of protein secondary structure (Boublik *et al.*, 1970; Fasman *et al.*, 1970) promoted by the high proline content, would be conducive to strong interaction with DNA (Bustin and Cole, 1970). The amino-terminal portion of lysine-rich histone, rich in acidic and hydrophobic residues and relatively poor in positive charge, may be specifically involved in another function (possibly interaction with a protein needed for derepression, chromatin cross-linking, or enzymatic reaction; Bustin and Cole, 1970). Or perhaps the function of the N-terminal segment is to moderate the effect of the C-terminal end by folding back on it after attachment to DNA.

In the present investigation, a comparison has been made between the interaction of DNA with intact f-1 and with its two cleavage products, incorporated separately and together into complexes with DNA. If DNA could bind strongly at many sites along the C-terminal piece of lysine-rich histone, as is suggested by its amino acid composition, then this fragment might be expected to produce more dramatic changes in the DNA circular dichroic spectrum than would the N-terminal polypeptide. The experiments described here show that this is the case. The isolated N-terminal third of the molecule, although it bound to DNA, failed to cause significant change in DNA circular dichroism upon complexation. On the other hand, the C-terminal fragment effected even larger changes than did equivalent amounts of whole f-1. When both cleavage products were bound to DNA, the N-terminal piece augmented the interaction of the C-terminal fragment with DNA, and enhanced its effect upon the circular dichroism of DNA. This result is in contrast to the role which the N-terminal portion of f-1 apparently plays in the intact f-1 molecule, where it decreases the conformational effect of the C segment upon DNA.

Materials and Methods

Histone Preparation and Cleavage. The lysine-rich (f-1) histone fraction from calf thymus was prepared by Method I of Johns (1964). The protein (100 mg) was further purified by chromatography on a Bio-Rex 70 (200–400 mesh, from Bio-Rad) column (2 × 27 cm), eluted with guanidinium chloride (Kinkade and Cole, 1966). The flow rate was 20 ml/hr. The effluent was monitored by its absorbance at 230 nm. Early fractions containing nonhistone contaminants and partially degraded histone were discarded, and fractions containing the lysine-rich histone were pooled and dialyzed against water. No attempt was made to isolate individual lysine-rich histone components. The protein solution was concentrated to 30 ml (on a rotary evaporator), and f-1 was precipitated with trichloroacetic acid, washed, and dried (Adler *et al.*, 1971). The yield was 63 mg.

Purified f-1 histone (trichloroacetate salt, 1.2 μmoles, 37.8 mg) was bisected with *N*-bromosuccinimide (Bustin and Cole, 1969b). Upon completion of reaction the mixture was concentrated to 2 ml and applied to a Sephadex G-100 column (2.8 × 176 cm) in 5% sucrose. Peptides were eluted at room temperature with 0.02 M HCl + 0.2% chloroform at a flow rate of 14 ml/hr (Bustin and Cole, 1969b). Peptides were detected by their absorbance at 230 nm; the modified tyrosyl of the N-terminal peptide was monitored by absorbance at 260 nm. The elution profile is given in Figure 1. The pooled peptide fractions were concentrated, precipitated with 20% trichloroacetic acid, and dried as above. The yield was 10.3 mg of peptide C, and 6.6 mg of N.

Phosphorylated f-1 was obtained as described previously (Adler *et al.*, 1971) by incubation with calf liver histone kinase HK_1 (Langan, 1968; Meisler and Langan, 1969) followed by chromatography and isolation as above. This preparation, containing 33.5 nmoles of P/mg of histone (= 0.70 P/mol wt 21,000), was subjected to *N*-bromosuccinimide cleavage (Bustin and Cole, 1969b). The alkali-labile phosphate content of the resulting N-terminal fragment was 0.53 phosphate group per mole (mol wt 6000). A control sample of f-1 was carried through the same incubation, isolation, and bisection procedures as the phosphorylated f-1, except that ATP was omitted from the enzymatic incubation mixture. This material was generously supplied by Dr. Thomas A. Langan.

f-1, its two fragments, and mixtures of the fragments were examined by 15% polyacrylamide disc gel electrophoresis (Bonner *et al.*, 1968) in the absence of urea. Gels were 20 cm long; 5 or 10 μg of each polypeptide was applied. Each poly-

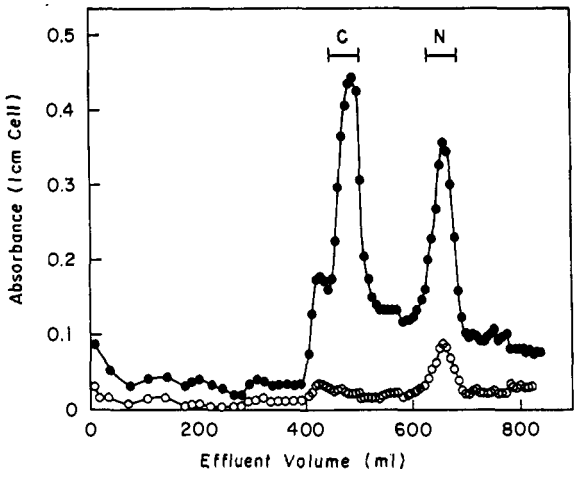

FIGURE 1: Fractionation of *N*-bromosuccinimide-reacted calf thymus f-1 histone. Column (2.8 × 176 cm) packed with Sephadex G-100, eluted with 0.02 M HCl + 0.2% $CHCl_3$. Flow rate 14 ml/hr; 7-ml fractions collected. Peptides detected by absorbance at 230 nm (●) and at 260 nm (○). Labels indicate tubes pooled for isolation of fragments C and N. Unreacted f-1 is eluted at 430 ml.

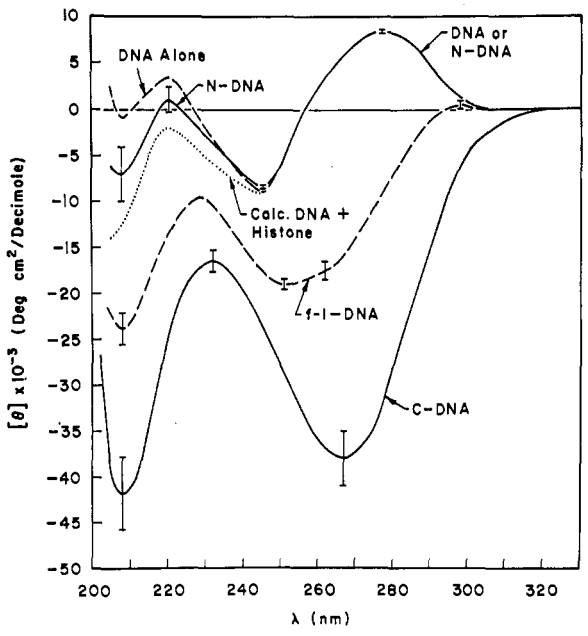

FIGURE 2: Circular dichroism spectra of complexes reconstituted from DNA and f-1 (or its fragments) at $r = 1.0$. r is the concentration ratio of histone (mole of peptide residues) to DNA (mole of nucleotide residues). N and C indicate the N-terminal and C-terminal f-1 fragments, respectively. Complex concentration = 0.75 to 1.0 × 10^{-4} M nucleotide residues, path length 1 cm, temperature 23°. Solvent 0.14 M NaF + 0.002 M Tris (pH 7.0). The circular dichroism of DNA alone is given for comparison; the curve "calculated DNA + histone" is the calculated sum of isolated DNA and f-1 (or C or N fragment) contributions (at $r = 1.0$) in the same solvent; both of these curves merge with that of N–DNA complex at wavelengths above 250 nm. Error bars indicate reproducibility of data in duplicate experiments.

peptide travelled homogeneously. Running time was 10 hr.

Stock solutions of f-1 and of its cleavage products (N and C fragments), concentrations ~3 × 10^{-3} M peptide residues, were prepared, stored frozen, and assayed by a modified biuret method as described previously (Adler *et al.*, 1971).

DNA and Other Materials. The same calf thymus DNA preparation was utilized as for previous studies (Adler *et al.*, 1971); its median molecular weight was 12.7 × 10^6. DNA concentrations were determined from OD_{258}, using ϵ_{258} (mole of residues) 6.8 × 10^3.

N-Bromosuccinimide was obtained from Eastman Kodak, sodium dodecyl sulfate and pyronin Y from Matheson Coleman & Bell. Guanidine hydrochloride was either purchased from Heico Inc., or was prepared from Eastman guanidine carbonate by the method of Nozaki and Tanford (1967). Inorganic salts and tris were reagent grade. Water was redistilled from glass.

Histone–DNA Complexes. Complexes of DNA with f-1 or with its fragments (N and/or C) were prepared by mixing these components, at the desired concentration and ratios, in a high-salt, dissociating, medium (2 M NaCl + 0.002 M Tris, pH 7) of total volume 2 ml, and then performing stepwise gradient dialysis at 4° (against 0.4 M NaF + 0.002 M Tris for 5 hr, 0.3 M NaF + 0.002 M Tris for 4 hr, and finally 0.14 M NaF + 0.002 M Tris for 16 hr; Fasman *et al.*, 1970). Dialysis tubing (Union Carbide 8) was first treated with acetic anhydride at room temperature for 1 day, washed, neutralized, and stored in 30% glycerol (Shih and Bonner, 1970). Complexes were not centrifuged. This dialysis method ensures reproducible reconstitution of complexes.

Concentrations of DNA in histone–DNA complexes were obtained by addition of 5% (w/v) sodium dodecyl sulfate to a final concentration of 0.1%. This treatment dissociates the complexes, and allows determination of DNA concentration by measurement of OD_{258} (ϵ_{258} 6.8 × 10^3). (This method agrees with perchloric acid hydrolysis, Adler *et al.* (1971), to within ±2% for complexes where both analyses were performed.) Concentrations of complexes were usually 0.75–1.0 × 10^{-4} M DNA nucleotide residues. Histone:DNA ratios (r) are reported as moles of histone (f-1, N, or C fragment) peptide residues per mole of DNA nucleotide residues, and were determined from the input concentrations.

Optical Measurements. Circular dichroism experiments were performed at 23° on a Cary 60 spectropolarimeter equipped with a 6001 CD attachment. The path length for most histone–DNA complexes was 1 cm; fused quartz cells (Optical Cell Co.) were used; OD_{260} was about 0.6. The half-bandwidth was set for 1.5 nm. The time constant was usually 3 sec, sometimes 10 sec (at low wavelength). Mean residue ellipticity, $[\theta]$, is reported in (deg cm²) per dmole of nucleotide residues in the complexes. Typical average errors in $[\theta]$ are indicated in Figure 2, and were based on duplicate experiments. The precision in band wavelength was ±0.5 nm. Ultraviolet spectroscopy was carried out on a Cary 14.

Centrifugation of Complexes. The extent of binding of f-1 and its fragments to DNA was determined by high-speed centrifugation of complexes. Complexes of f-1–DNA, N–DNA, and C–DNA were prepared, containing 6 μmoles each of peptide and nucleotide residues in 6 ml of 0.14 M NaF (no Tris; 10^{-3} M complex, $r = 1.0$). Five milliliters of each complex, of DNA, and of each polypeptide component (f-1, C, or N, dialyzed separately, 10^{-3} M peptide) were spun for 22 hr at 5° in a Spinco Model L centrifuge at 130,000*g*. The pellets (redissolved in 3 ml of 0.01 M NaOH), supernatants (top half), and aliquots of the original unspun solutions were analyzed for DNA (by perchloric acid digestion; Adler *et al.*, 1971) and for protein. In the absence of DNA (in supernatants and in protein solutions) histone was determined by ultra-

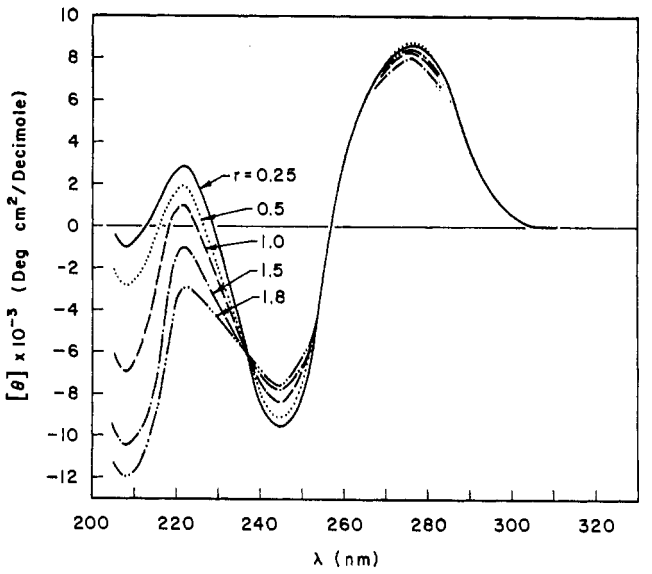

FIGURE 3: Circular dichroism of fragment N–DNA complexes at various values of *r*. Same conditions as in Figure 2.

violet absorbance (ϵ_{220} = 810 ±20 per mole peptide for f-1, C, and N polypeptides). In addition, protein was assayed, in all cases, by a biuret procedure (Zamenhof, 1957) performed in the presence and absence of $CuSO_4$, in order to correct for DNA absorbance.

Results

Physical Characteristics of the Polypeptide Fragments. The chromatographic elution profile (Figure 1) obtained for calf thymus lysine-rich (f-1) histone after cleavage with *N*-bromosuccinimide was very similar to that observed by Bustin and Cole (1969b) for rabbit thymus f-1. The C-terminal fragment, C (N_1 in Bustin and Cole's nomenclature), was cleanly resolved from unreacted f-1 and from the N-terminal third of the molecule, N (N_2). The position of the N component was confirmed by the absorbance peak at 260 nm, indicative of the modified tyrosyl residue.

The f-1 preparation and each of its fragments appeared homogeneous by polyacrylamide gel electrophoresis (Bonner *et al.*, 1968). Both polypeptides, N and C, migrated 7.2 cm, compared to 5.2 cm for whole f-1, and 13.4 cm for a low molecular weight marker, pyronin Y. Mixtures of N and C fragments in various amounts yielded a single band at the position of N or C polypeptide alone. Mixtures of f-1 and N fragment migrated independently. Even in the absence of urea there is no evidence of strong specific interaction or aggregation for either mixture; the combination of N and C fragments does not have the electrophoretic properties of the intact f-1 molecule, nor do any slow-moving or diffuse bands appear.

Circular dichroic spectra of intact f-1 and of N and C fragments (all at 10^{-3} M peptide residues, 1-mm cell) were identical, within experimental error. All spectra displayed a negative shoulder at 223 nm and a negative band $[\theta]_{200}$ = −19,000, characteristic of the highly cationic, randomly coiled f-1 conformation (Fasman *et al.*, 1970). An equimolar mixture of N and C fragments, in such a proportion as to reconstruct the f-1 chain (0.29×10^{-3} M peptide residues N + 0.71×10^{-3} M peptide residues C) yielded this same circular dichroism pattern. This finding shows that no gross conformational change occurs in the polypeptide chains when fragments N and C are recombined in the absence of DNA. Both this and the electrophoresis data indicate that N- and C-cleavage products in a mixture act independently.

Circular Dichroism of Histone–DNA Complexes Formed with f-1 or Its Fragments. The near-ultraviolet circular dichroism spectrum of native calf thymus DNA is characterized by positive ($[\theta]_{277}$ = 8400) and negative ($[\theta]_{245}$ = −8900) ellipticity bands of nearly equal intensity. The calculated sum of the circular dichroism spectra for isolated DNA and calf thymus f-1 (or N or C polypeptides), as well as the circular dichroism of DNA alone, are given in Figure 2; this calculation was made for a histone:DNA ratio, *r* (moles of peptide residues: moles of nucleotide residues), of 1.0.

A comparison of histone–DNA complexes derived from whole f-1, the cleaved N-terminal third of the molecule, and the isolated C-terminal remainder of the protein is given in Figure 2. All complexes are at 10^{-4} M nucleotide concentration and an equal peptide residue concentration (r = 1.0). Under these conditions fragment N produces very little change in the DNA circular dichroism. On the other hand, complex formation with fragment C causes even greater distortion of the DNA circular dichroism spectrum than does DNA attachment to the intact f-1 molecule. Very similar results were obtained with 10^{-3} M complexes.

As the peptide residue:nucleotide residue ratio, *r*, is increased from 0 to 1.2, the same series of progressive changes in the DNA circular dichroism spectrum is observed for C–DNA complexes as for f-1–DNA complexes (Fasman *et al.*, 1970). The only difference is that, at any given value of *r* (such as r = 1.0 in Figure 2), the distortions are greater for C–DNA than for f-1–DNA.

However, as the ratio, *r*, is increased from 0 to 1.8 for N–DNA complexes (Figure 3), only minor changes are observed in the DNA circular dichroism. The differences in the ellipticity value of the 277-nm positive band are negligible, and show no trend. The spectral changes below 240 nm can be attributed to the increase in protein circular dichroism contribution. Thus, the relatively lysine-poor N-terminal polypeptide, N, by itself, is not capable of producing, at any ratio, the same type of circular dichroism distortion as native f-1 or as the lysine-rich C-terminal fragment, C. Enzymatic phosphorylation of polypeptide N at Ser-37 does not affect the rotational properties of N–DNA complexes at any ratio.

This lack of circular dichroism change due to the N piece could not be attributed to loss of or aggregation of fragment N during dialysis. Since fragment N is of lower molecular weight (6000) and lower lysine content (19%) than component C or whole f-1, the N polypeptide might conceivably pass through dialysis tubing or form aggregates as the salt concentration is lowered during dialysis (as do the arginine-rich and slightly lysine-rich histone fractions at moderate salt concentrations (Edwards and Shooter, 1969)), thus preventing binding to DNA. However, the N fragment was found (by biuret analysis) to remain in solution within the dialysis bag during dialysis both in the absence and presence of DNA. Furthermore, the fact that the N fragment remained in the supernatant after dialysis followed by high-speed centrifugation of the peptide solution (see later) indicated that aggregation was not occurring. In order to investigate this point further, a gradient dialysis of a N–DNA complex (r = 1.8) was performed in the presence of 5 M urea. (This treatment is utilized to prevent arginine-rich histone aggregation and to ensure formation of complexes (Shih and Bonner, 1970; Shih and Fasman, 1971.)) The circular dichroism spectrum of

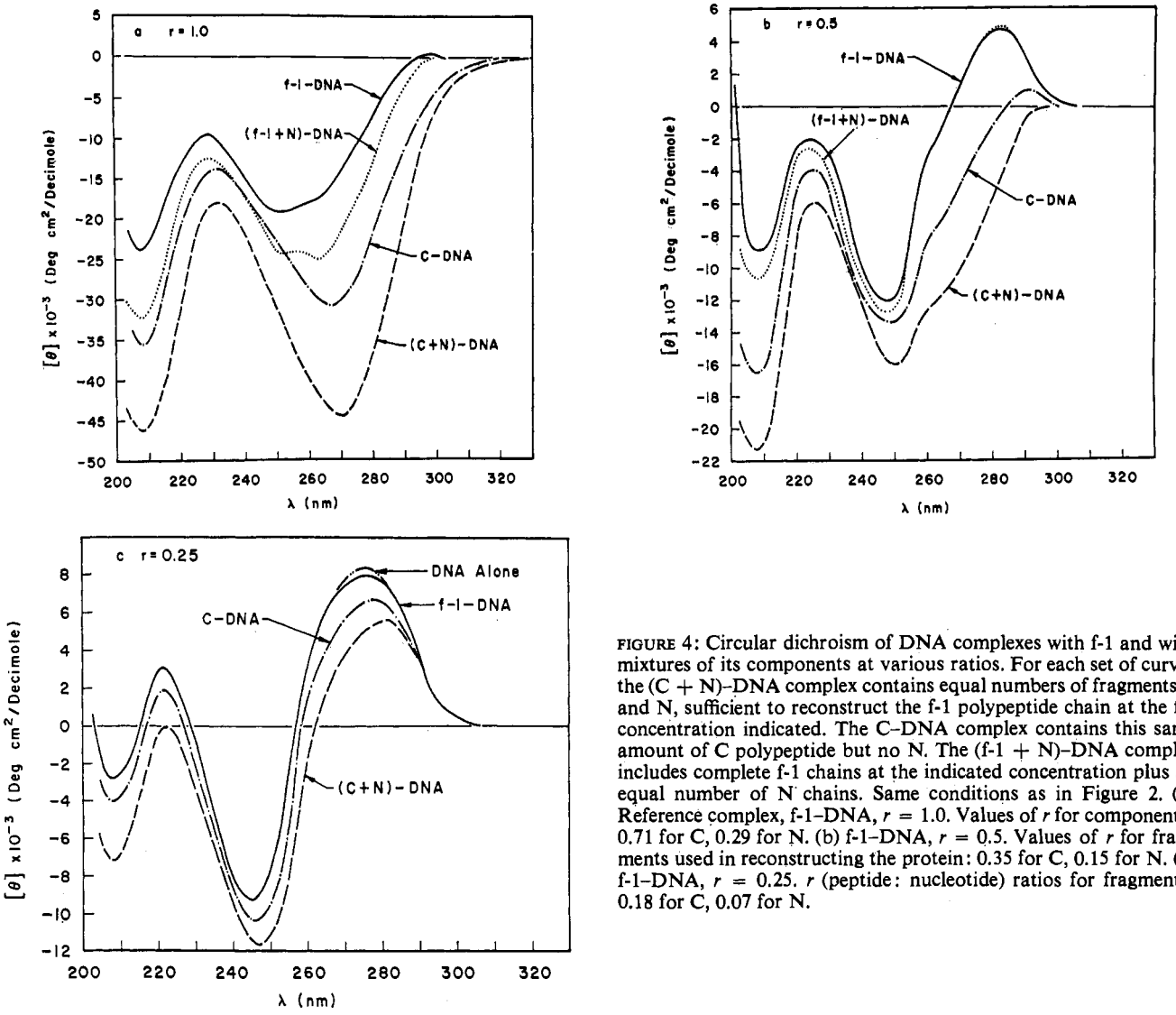

FIGURE 4: Circular dichroism of DNA complexes with f-1 and with mixtures of its components at various ratios. For each set of curves the (C + N)–DNA complex contains equal numbers of fragments C and N, sufficient to reconstruct the f-1 polypeptide chain at the f-1 concentration indicated. The C–DNA complex contains this same amount of C polypeptide but no N. The (f-1 + N)–DNA complex includes complete f-1 chains at the indicated concentration plus an equal number of N chains. Same conditions as in Figure 2. (a) Reference complex, f-1–DNA, $r = 1.0$. Values of r for components: 0.71 for C, 0.29 for N. (b) f-1–DNA, $r = 0.5$. Values of r for fragments used in reconstructing the protein: 0.35 for C, 0.15 for N. (c) f-1–DNA, $r = 0.25$. r (peptide: nucleotide) ratios for fragments: 0.18 for C, 0.07 for N.

this N–DNA complex was identical to that of the complex formed in the absence of urea. Therefore, loss of fragment N through dialysis or aggregation was not responsible for its ineffectiveness at causing circular dichroism change, apparent in Figure 3. Furthermore, as will be shown later, cleaved polypeptide N does bind to DNA, although somewhat less strongly than do fragment C or intact f-1.

A series of experiments was performed to determine whether the differences in circular dichroism between f-1–DNA and C–DNA complexes (Figure 2) reflected only the difference in lysine composition between the two molecules. Three sets of f-1–DNA, C–DNA, and N–DNA complexes (at 10^{-4} M nucleotide) were prepared. In the first set each complex contained the same number of lysine residues (not total peptide residues) as is present in f-1–DNA, $r = 1.0$. The second set was matched for lysine content in C–DNA, $r = 1.0$; the third set for N–DNA, $r = 1.0$. In the first set, for example, all of the complexes have a lysine:nucleotide ratio of 0.29. That is, the negative charge on the DNA is 30% neutralized by cationic peptide residues (including the small amount of arginine present in lysine-rich histone). The total peptide:nucleotide ratios, r, for these complexes are 1.53 for N–DNA, 1.00 for f-1–DNA, and 0.89 for C–DNA. The circular dichroism spectrum for N–DNA is given in Figure 3, that for f-1–DNA in Figure 2; the data for this C–DNA complex are very similar to the C–DNA curve shown in Figure 4a. The circular dichroism spectra for C–DNA and f-1–DNA are not identical: fragment C produces greater perturbation of DNA conformation than does the complete f-1 molecule, even when the peptide concentrations are adjusted to yield the same amount of lysine. The same conclusion was reached from the other two sets of data. Thus, the sequence of effectiveness in causing DNA distortion, C > f-1 >> N, is not entirely a function of the percentage lysine in each polypeptide.

Circular Dichroism of Histone–DNA Complexes Formed with Mixtures of Fragments. The f-1 chain was formally reconstituted by mixing equimolar amounts of N-terminal and C-terminal fragments, and the effect of this mixture on DNA was examined. Histone–DNA complexes were formed by gradient dialysis with these polypeptide mixtures at various values of r (total peptide:nucleotide ratio), and the circular dichroism spectra were compared to those of normal f-1–DNA complexes at the same values of r. The results are shown in Figure 4 for $r = 1.0$, 0.5, and 0.25. In each case the (C + N)–DNA complex exhibits greater deviation from DNA structure than does the comparable f-1–DNA complex, even though the same types and numbers of amino acid residues are present in both complexes. Thus, a mixture of N and C fragments

has neither the same physical properties (see above) nor the same interaction with DNA as does the intact f-1 protein.

In addition, Figure 4 indicates that the C-terminal fragment alone produces a greater circular dichroism change than does an equimolar amount of the entire f-1 chain, even though the C portion contains only 71% of the peptide residues (and 79% of the lysine residues) of f-1. This is consistent with the results of the experiments performed at constant lysine concentration.

An unexpected finding is that, although the cleaved N polypeptide alone causes negligible circular dichroism distortion when complexed to DNA, it greatly enhances the changes brought about by the C fragment. That is, Figure 4 shows that, when both cleavage products are present, (C + N)–DNA complexes are characterized by more circular dichroism change than are C–DNA complexes containing the same amount of C fragment, although N fragment alone has no effect at $\lambda > 250$ nm (Figure 3).

Examination of N polypeptide phosphorylated at Ser-37 does not alter this result; (C + N)–DNA complexes formed with phosphorylated N fragment yielded circular dichroism spectra identical (within experimental error) to those reconstituted with control N polypeptide (derived from f-1 incubated with kinase but no ATP). For example, at $r = 1.0$ (same definition as in Figure 4) both of these (C + N)–DNA complexes were characterized by $[\theta]_{268} = -43{,}000 \pm 4000$; at $r = 0.5$ the complexes (phosphorylated and control) both displayed bands at $[\theta]_{287} = 1000$, negative shoulders at 262 nm, and $[\theta]_{248} = -15{,}500 \pm 300$.

A set of (C + 3N)–DNA complexes was reconstituted, containing the same amounts of C fragment but three times the amount of N fragment used for the complexes of Figure 4. Major features of the resulting circular dichroism spectra were $[\theta]_{271} = -54{,}500$ at $r = 1.0$; a double negative band, $[\theta]_{252} = -19{,}200$, $[\theta]_{262} = -17{,}600$ at $r = 0.5$; and $[\theta]_{283} = +4400$ at $r = 0.25$. Thus, incorporation of excess N fragments into complexes causes additional circular dichroism change.

Centrifugation of Histone–DNA Complexes. The extent of binding of f-1 and its fragments to DNA was determined by high-speed centrifugation of the complexes. Under the conditions used, control samples of N or C fragments alone (after gradient dialysis into 0.14 M NaF) were found in the supernatant (>96%, indicating absence of aggregation), whereas DNA alone was completely pelleted (>99%). In the case of all three complexes (f-1–DNA, N–DNA, and C–DNA at $r = 1.0$), only 0.4% of the DNA was present in the supernatant therefore, the histone–DNA complexes pelleted. Amounts of free protein in the supernatants were found to be 8% of the starting material for f-1–DNA and C–DNA, and 30% for the N–DNA complex. (The remainder of the DNA and proteins was found in the pellets.) Therefore, the N fragment binds less completely (70%) to DNA than does whole f-1 or the C fragment (>90% each) in complexes reconstituted at a peptide:nucleotide ratio of 1. However, since most of the N fragment is incorporated into the complex, this difference in binding affinity cannot account for the lack of circular dichroism change in N–DNA complexes.

Discussion and Conclusions

Formation of complexes between f-1 histone and DNA by means of gradient dialysis annealing causes progressive distortion of the DNA circular dichroism spectrum as increasing amounts of histone are included into the f-1–DNA complex (Fasman *et al.*, 1970). This distortion has been interpreted as an indication of alterations in the asymmetric environment of the DNA nucleotide chromophores.

One major conclusion to be drawn from the present study of DNA complexes with f-1-cleavage products is that the C-terminal, lysine-rich fragment binds more strongly to DNA, and with a much greater capability to distort the DNA circular dichroism spectrum, than does the N-terminal fragment. Therefore, in intact lysine-rich histone (f-1) the C-terminal part of the molecule is probably the chief site of electrostatic binding to DNA, and is primarily reponsible for the structural changes observed in DNA upon combination with histone. It is not yet resolved whether these changes are conformational in nature, or whether they result from supercoiling of the DNA (Pardon and Richards, 1971).

On the other hand, the cleaved N-terminal third of f-1, although it binds to DNA to nearly the same extent as does the C fragment (70% as opposed to 92%) is, by itself, incapable of causing a significant change in the DNA circular dichroic spectrum. This result shows that the N fragment (which is a basic polypeptide) can interact with DNA, but not in such a manner as to change the structural state of the DNA. The amino-terminal segment of intact f-1 may be involved in functions other than electrostatic binding to DNA, such as folding back on the C-terminal segment, thus modifying the latter's effect. The amount of circular dichroic distortion produced upon reconstitution of complexes with DNA is not simply dependent upon the amount of lysine in the complex. The differences in interaction with DNA found among C and N fragments and intact f-1 are more specific than that, and are probably related to amino acid sequence and/or secondary structure.

In an analogous study Li and Bonner (1971) bisected the slightly lysine-rich, serine-rich histone IIb2 (f2b) by means of cyanogen bromide. This protein, whose primary structure is known (Iwai *et al.*, 1970), has most of its basic amino acids clustered toward the N terminus (opposite from f-1). DNA complexes formed with IIb2–N half-molecules have melting temperatures 13° higher than those of IIb2–C complexes (Li and Bonner, 1971). Furthermore, these two T_M values could be correlated with biphasic melting curves of native nucleohistone (Li and Bonner, 1971) and of complexes reconstituted with various histones (Ansevin and Brown, 1971). Thus, evidence is accumulating that different portions of histone molecules interact to varying extents with DNA, and may well have different roles in chromatin.

When both C and N fragments of f-1 are incorporated together into complexes, the observed circular dichroism curves (Figure 4) are not those expected for the sum of the effects of isolated C and of N fragments. (Since N alone does not significantly affect the circular dichroic spectrum of DNA, it might not be expected to influence the spectra of C–DNA complexes.) Instead, the data show that the N fragment modifies the interaction of C with DNA in such a way as to augment the circular dichroism changes. The N polypeptide, when cleaved off, may simply be incorporated into the optically active structure of the C–DNA complex as it is being formed. In this respect the N fragment may act in a manner similar to ammonium and guanidinium cations, whose presence greatly enhances circular dichroism distortion of f-1–DNA complexes (Adler and Fasman, 1971), but which have no effect upon DNA itself. There is no reason to assume that the two cleavage products do not bind independently and randomly onto DNA; at $r = 1$ the charge on DNA is only 30% neutralized by histone, and there is sufficient DNA available for combination with both N and C fragments. N fragments cannot be binding

onto specific sites to complete the f-1 chain, since data on (f-1 + N)–DNA and (C + 3N)–DNA complexes show that the N fragment exerts a fairly nonspecific synergistic effect when it is severed from the C portion of f-1. Furthermore, the decrease in DNA circular dichroism distortion occurring when the serine-37 position of f-1 is phosphorylated (Adler *et al.*, 1971) is lost when the phosphorylated N fragment is bound to DNA, either alone or in combination with the C fragment.

Another significant conclusion is that the intact f-1 histone chain combines with DNA in a specific manner, and not merely as the sum of its parts. The evidence for this is that (C + N)–DNA complexes (formed with enough of each peptide to reconstruct the f-1 molecule formally) do not behave optically like the analogous f-1–DNA complexes formed with intact f-1. The N-terminal segment of intact f-1 appears to cause a decrease in the conformational effect upon DNA when binding to the C-terminal segment occurs. Perhaps in native chromatin the C-terminal section of f-1 is strongly attached to DNA (mainly through electrostatic interaction), while the N-terminal portion is partially peeled away from the DNA (and possibly folded back onto the C segment) so that it cannot interfere with proper site binding of the basic end of the histone. This type of binding would account for the difference between f-1–DNA and (C + N)–DNA complexes, and for the moderating effect of the N portion of f-1 when it is covalently linked to the C segment. Such a folded tertiary structure for f-1 when bound to DNA would also explain the manner in which phosphorylation of intact f-1 (but not of the N fragment alone) could alter the effect upon DNA conformation of f-1 binding. Thus, fragment N alone binds to DNA in a completely different manner than it does when it is part of intact f-1. (Perhaps, in addition, the strikingly nonbasic peptide region in the center of the f-1 molecule (the amino end of polypeptide C; Bustin and Cole, 1970) might be only loosely attached to DNA.) The weakly bound regions of histone might then be available for specific functions involving other proteins or RNA (Bustin and Cole, 1970). This speculation is consistent with the finding (Bustin and Cole, 1969b, 1970) that amino acid differences among the subfractions of f-1 probably occur primarily in the lysine-poor N fragment.

In summary this study has shown that when f-1 is cleaved chemically, fragment C is the only portion of f-1 which can cause circular dichroic change in DNA upon binding. Fragment N is capable of attachment to DNA, although any binding specificity residing in this part of the f-1 chain appears to be lost upon cleavage. These facts may be added to the knowledge that the phosphorylated N fragment cannot be distinguished from the normal N fragment in its binding to DNA when recombined with the isolated C fragment. However, in the intact f-1 molecule phosphorylation does alter the influence of this histone upon DNA conformation. These results are highly suggestive of a model wherein the N segment in native f-1 partially folds back on the C segment and modifies the binding of the C-terminal portion of f-1. Histone folding is consistent with the results of Clark and Felsenfeld (1971) which showed that histones are not distributed evenly along the DNA double helix in chromatin. Thus, polypeptide N–polypeptide C interactions could modify the conformational alterations in DNA produced by histone binding.

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