Interactions of H1 and H5 Histones with Polynucleotides of B- and Z-DNA Conformations†

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ABSTRACT: Interactions of chicken H1 and H5 histones with poly(dA-dT), poly(dG-dC), and the Z-DNA structure brominated poly(dG-dC) were measured by a nitrocellulose filter binding assay and circular dichroism. At low protein:DNA ratios, both H1 and H5 bound more Z-DNA than B-DNA, and binding of Z-DNA was less sensitive to interference by an increase in ionic strength (to 600 mM NaCl). H5 histone bound a higher percentage of all three polynucleotides than did H1 and caused more profound CD spectral changes as well. For spectral studies, histones and DNA were mixed in 2.0 M NaCl and dialyzed stepwise to low ionic strength. Prepared in this way or by direct mixing in 150 mM NaCl, complexes made with right-handed poly(dG-dC) showed a deeply negative ψ spectrum (deeper with H5 than with H1). Complexes of histone and Br-poly(dG-dC) showed a reduction in the characteristic Z-DNA spectral features, with H5 again having a greater effect. Complexes of poly(dA-dT) and H5, prepared by mixing them at a protein:DNA ratio of 0.5, displayed a distinctive spectrum that was not achieved with H1 even at higher protein:DNA ratios. It included a new negative band at 287 nm and a large positive band at 255 nm, giving the appearance of an inverted spectrum relative to spectra of various forms of B-DNA. These findings may reflect an ability of the different lysine-rich histones to cause varying conformational changes in the condensation of chromatin in DNA regions of highly biased base sequence.

Studies of synthetic DNA analogues have demonstrated considerable polymorphism in the basic structure of the double helix. These include structural differences between the alternative helix families, right-handed A-DNA and B-DNA and left-handed Z-DNA, differences among B-DNA variants such as poly(dA-dT) or poly(dG-dC), and nonuniformity within helical oligonucleotides of mixed base composition (Pohl & Jovin, 1972; Wang et al., 1979; Wells et al., 1980; Dickerson et al., 1982; Arnott et al., 1983). These findings lead to the proposal that structural variations in DNA resulting from particular sequences may provide recognition sites for protein–DNA interactions and may play an important role in the utilization of genetic information.

In eukaryotic cells, DNA is associated with a variety of proteins. When associated with histones, DNA becomes coiled around a core of histones H2A, H2B, H3, and H4, forming the organized structure of nucleosomes, and the lysine-rich histones bind to a linker DNA of variable length between the nucleosome cores [reviewed in McGhee & Felsenfeld (1980) and Igo-Kemenes et al. (1982)], serving to condense the chromatin in formation of higher order structure (Thoma & Koller, 1981). The lysine-rich histones comprise a heterogeneous group of proteins, with much more amino acid sequence variation than the core histones (Von Holt et al., 1979). The different types of lysine-rich histones include subfractions of H1, which may occur in different proportions in different tissues (Bustin & Cole, 1968), and more distinct variants such as H5, characteristic of avian nucleated erythrocytes (Neelin et al., 1964) or H1°, an analogous protein in mammalian cells (Panyim & Chalkley, 1969). Switches in the expression of genes coding for these lysine-rich histones have been found to occur during sea urchin development (Newrock et al., 1977), spermatogenesis (Seyedin & Kistler, 1979), and avian erythropoeisis (Mura et al., 1982). In addition, H1° occurs selectively in cells that are nonreplicating (Panyim & Chalkley, 1969); a marked increase in its quantity occurs during induction of differentiation (and reduction of replication) of cultured cell lines (Keppel et al., 1979). The occurrence of H5 is characteristic of cells that are inactive in both transcription and replication (Billet & Hindley, 1972; Urban et al., 1980).

The above findings of different functional associations of the H1 and H5 histones suggest that they may interact differently with DNA as they act to condense chromatin. As these differences could involve recognition of local variations in DNA conformation, we have examined the interaction of the H1 and H5 histones with the synthetic polymers poly(dA-dT), poly(dG-dC) in the B-DNA form, and brominated poly(dG-dC), which is stabilized in the Z form (Möller et al., 1984). Both histones bound all three polymers and caused changes in the circular dichroism spectra of the polynucleotides; however, they differed in the extent of binding, the sensitivity of binding to ionic strength, and the type or extent of CD spectral change induced with the various forms of DNA. The major effect on the spectra of the B form of poly(dG-dC) was the induction of a deeply negative CD band, characteristic of a ψ spectrum (Jordan et al., 1972). The effect on the Z form of poly(dG-dC) was toward reduction in the Z-like characteristics of the spectrum. A very different spectrum was induced in poly(dA-dT)–histone H5 complexes, characterized by a new negative band centered at 287 nm and intensification of a positive band at 255 nm. In all three cases, H5 had a larger effect than H1.

Materials and Methods

Poly(dG-dC) and poly(dA-dT) were purchased from P-L Biochemicals, Inc. Deoxyribonuclease I and DNA polymerase for nick translation were obtained from Boehringer Mannheim. Nitrocellulose paper, pore size 0.45 μm, was a product of Schleicher & Schuell. Bromination of poly(dG-dC) was done according to the method of Möller et al. (1984). Polynucleotides were radioactively labeled by the nick translation technique described by Rigby et al. (1977).

Histone H5 was prepared from erythrocytes of adult white leghorn chickens as described previously (Mura et al., 1974). Histone H1 and core histones were prepared from calf thymus by the procedure of Johns (1964). Lysozyme was purchased

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from Sigma. The purity of histones was analyzed by the sodium dodecyl sulfate (SDS) gel electrophoresis procedure of Thomas & Kornberg (1975). Protein concentration was determined by the method of Lowry et al. (1951). Poly(dA-dT) was fractionated on a Sepharose 4B column, with 100 mM NaCl–5 mM Tris–1 mM EDTA, pH 7.6, as the eluting buffer. Polynucleotides were characterized with respect to molecular weight by electrophoresis on 6% polyacrylamide slab gels by the method of Peacock & Dingman (1967). Gels were stained with ethidium bromide at 0.5 μg/mL in H_2O and photographed under UV illumination. Calibration markers consisted of *Hae*III or *Hae*II restriction enzyme fragments of $\phi X174$ DNA. Poly(dG-dC) had a size distribution from 0.87 to 2.7 kb, Br-poly(dG-dC) from 1.7 to 2.7 kb, poly(dA-dT) from 0.11 to 2.7 kb, fractionated high molecular weight poly(dA-dT) from 0.8 to 1.7 kb, and low molecular weight poly(dA-dT) from 0.1 to 0.35 kb.

Filter Binding Assay. The interaction between DNA and histones was measured by determining the protein-dependent accumulation of ^{3}H-labeled synthetic polynucleotides on nitrocellulose paper. Histones and synthetic polynucleotides were mixed in a total volume of 0.1 mL containing 5 mM Tris-HCl or 5 mM phosphate buffer, pH 7.6, in 0.15 M NaCl. The DNA and the histone concentrations are indicated in each experiment. Polynucleotide concentrations were determined from the radioactivity of samples of known specific activity. Histone solutions were prepared at known concentrations with weighed samples of dry histone and concentrations were confirmed by the Lowry procedure (Lowry et al., 1951). In a typical assay, the desired amount of histone was diluted into 40 μL of distilled water immediately before use. Ten microliters of DNA solution containing 50 or 100 ng of DNA was added to 50 μL of incubation buffer (2 times concentrated). DNA was added to the histones and the mixtures, in a total volume of 100 μL each, were incubated for 20 min at 25 °C. Then each mixture was diluted with 900 μL of buffer and immediately filtered through a nitrocellulose paper, which had been soaked previously in the buffer. The tubes were rinsed 3 times with 1 mL of the buffer mixture, and these solutions were passed through the filters. All operations were at room temperature. The filters were dried and counted in toluene-based scintillation fluid.

Circular Dichroism. Circular dichroism spectra were obtained in a J-20 Jasco model spectropolarimeter, which was routinely calibrated with a solution of *d*-10-camphorsulfonic acid in water. Quartz cuvettes of 1-cm path length were used, and samples were scanned between 320 and 245 nm. All measurements were made at 23 ± 1 °C.

Complexes of proteins and polynucleotides were made by step gradient dialysis at 4 °C following the modified procedure of Simpson & Kunzler (1979). Complexes were prepared by mixture of protein and polynucleotide in a solution at high ionic strength (2.0 M NaCl) followed by steps of dialysis against 1, 0.6, 0.3, 0.1, and 0.02 M NaCl in 10 mM Tris–10 mM EDTA, pH 7.6. The concentration of polynucleotides was determined by absorbance at 260 nm using the following extinction coefficients for 0.1% solutions: Br-poly(dG-dC), 14.4; poly(dG-dC), 18; poly(dA-dT), 17. For each complex 50 μg of polynucleotide was used with 25 μg of H5, 30 μg of H1, or 30 μg of lysozyme, unless indicated otherwise. Alternatively, the complexes of protein with polynucleotides were prepared by direct mixing of both components previously equilibrated at the final salt concentration and buffered with 5 mM Tris, pH 7.6. In all cases, the protein:nucleic acid ratios presented in the text refer to the amounts that were mixed; complexes were not separated from free components. For observation of the effect of ionic strength on CD spectra, fixed amounts of protein and polynucleotides were extensively dialyzed into 5 mM Tris, pH 7.6, and measured amounts of 6 M stock solution of NaCl were added to the initial mixture to obtain the desired final concentration of NaCl. Some of the complexes at higher protein and salt concentrations were slightly opalescent. Light scattering was measured as absorbance at 400 nm in a Zeiss spectrophotometer. The scattering at 400 nm varied from 2% to 15% of the absorbance at 260 nm. In repeated experiments with different preparations having light scattering values in this range, CD spectra were qualitatitively similar.

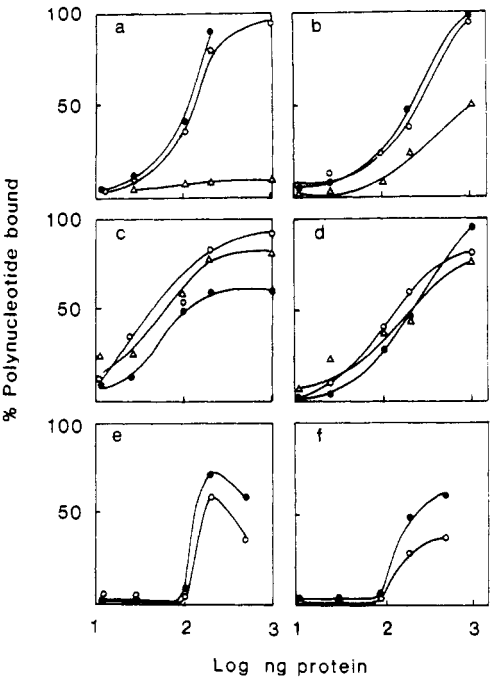

FIGURE 1: Binding characteristics of histones H5 (a, c, and e) and H1 (b, d, and f) to poly(dG-dC) (a and b), Br-poly(dG-dC) (c and d), and poly(dA-dT) (e and f). Increasing amounts of histones were added to 50 ng of ^{3}H-labeled polynucleotides in 5 mM Tris–NaCl buffer, pH 7.6, containing 30 (●), 150 (○), or 600 (▲) mM NaCl.

Results

Nitrocellulose Filter Retention. These experiments are based on the observation that when a dilute solution of histone is passed through a nitrocellulose filter, the histone is retained, whereas double-helical DNA alone passes through almost quantitatively. DNA is retained on the filter only when complexed with protein.

Both the right-handed poly(dG-dC) and the left-handed Z-DNA form, Br-poly(dG-dC), were bound by histones H1 and H5 (Figure 1). The binding depended on the concentration of both DNA and histone, as well as on ionic strength. To achieve a binding of 80–100% of a 50-ng sample of DNA, a 4–20-fold excess of histone was required. With lower amounts of protein, both histones bound more Br-poly(dG-dC) (Figure 1c,d) than unmodified polymer (Figure 1a,b).

The optimal ionic strength was higher for binding of Br-poly(dG-dC) than for binding of unmodified polymer, and the histone–Z-DNA interaction was much less sensitive to inhibition by increasing ionic strength (Figure 2a,b). Over a wide range of ionic strength, H1 and H5 were similar in their binding of Br-poly(dG-dC), whereas H5 was more effective than H1 in binding unmodified polymer (Figure 2a,b). In 20 mM NaCl, some nonspecific binding was observed with ly-

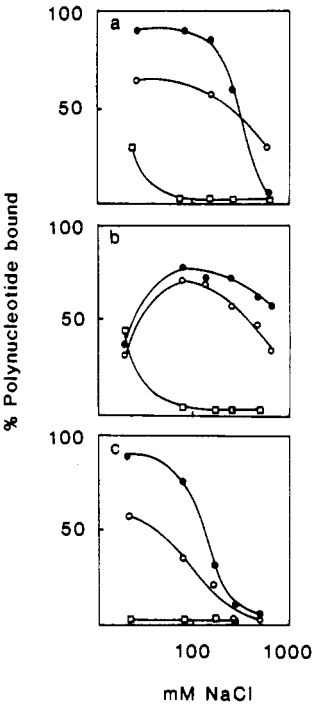

FIGURE 2: Filter retention of histone–polynucleotide complexes at different ionic strengths. (a) A mixture of 100 ng of ^{3}H-labeled poly(dG-dC) in 5 mM Tris, pH 7.6, at different NaCl concentrations was allowed to react with 300 ng of H5 (●), H1 (○), or lysozyme (□). (b) 50 ng of ^{3}H-labeled Br-poly(dG-dC) with 200 mg of H5 (●), H1 (○), or lysozyme (□). (c) 50 ng of ^{3}H-labeled poly(dA-dT) with 200 ng of H5 (●), H1 (○), or lysozyme (□).

sozyme as well as with the histones, but in 80 mM or higher NaCl concentrations, only the histones bound the DNA samples.

H1 and H5 histones both showed ionic strength dependent binding of poly(dA-dT) as well (Figures 1e,f and 2c). When 200 ng of protein and 50 ng of this polymer were mixed, H5 bound more of the polynucleotide over a wide range of ionic strengths but binding by both histones was very low at 300 mM or higher concentrations of NaCl. Lysozyme did not mediate binding of this polymer at these concentrations, even in 20 mM NaCl.

Circular Dichroism Spectra. To determine whether the histone–DNA interactions caused differing conformational changes in the DNAs, CD spectra of the protein–nucleic acid complexes were measured. These experiments take advantage of the fact that the spectra of H1 and H5, at the concentrations used, are nearly featureless above 250 nm, so that DNA spectral changes above 250 nm can be monitored without interference from the protein. In this region, poly(dG-dC) exhibits a typical B-DNA pattern, with a positive ellipticity band peaking at 270 nm and a negative band centered at 249 nm (Figure 3). Br-poly(dG-dC) shows the Z-DNA spectrum, with a negative band centered at 291 nm and a positive band peaking at 263–265 nm (Figure 4). Poly(dA-dT) exhibits a B-DNA spectrum, with a positive ellipticity band peaking at 258–260 nm and a negative band at 246–248 nm (Figure 5).

Complexes were prepared first by mixing of histones and poly(dG-dC) in 2.0 M NaCl followed by stepwise dialysis to low ionic strength and then adjustment to 150 mM NaCl. As increasing amounts of H5 were added to poly(dG-dC), the polynucleotide CD peak at 270 nm decreased and disappeared at a protein:polynucleotide ratio of 0.2; at higher ratios, it was replaced by a strong negative band that had a maximal value at 263 nm (Figure 3). With histone H1 and a protein:poly-

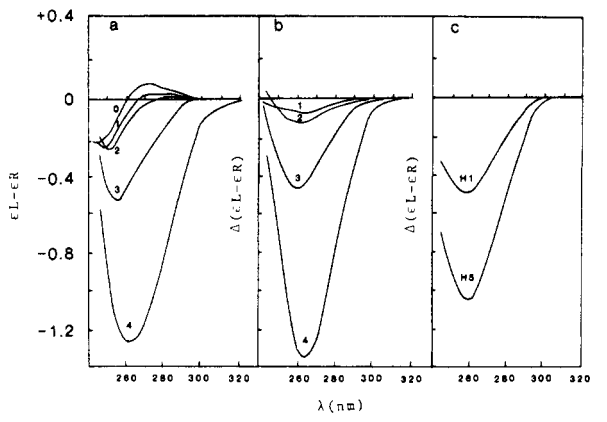

FIGURE 3: CD spectra of poly(dG-dC) and protein–poly(dG-dC) and calculated difference spectra. The solvent was 5 mM Tris–10 mM EDTA–150 mM NaCl, pH 7.6. (a) CD spectra as a function of an H5:polynucleotide ratio of 0.1 (line 1), 0.2 (line 2), 0.4 (line 3), and 0.5 (line 4); poly(dG-dC), line 0. (b) Calculated difference spectra as a function of H5:polynucleotide ratio: 0.1 (line 1); 0.2 (line 2); 0.4 (line 3); 0.5 (line 4). (c) Calculated difference spectra of H5–poly(dG-dC) at a ratio of 0.5 and H1–poly(dC-dC) at a ratio of 0.6 (w/w).

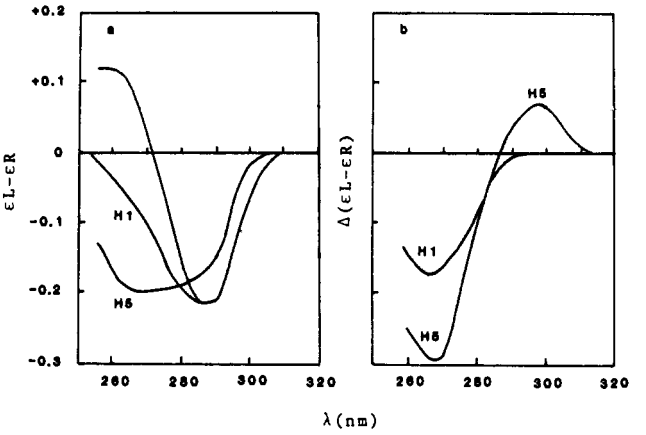

FIGURE 4: CD spectra of Br-poly(dG-dC) and protein–Br-poly(dG-dC) and calculated difference spectra. Solvent was 5 mM Tris–10 mM EDTA–150 mM NaCl, pH 7.6. (a) CD spectra of Br-poly(dG-dC) alone (unmarked line), H5–Br-poly(dG-dC) at a ratio of 0.5 (w/w) and H1–Br-poly(dG-dC) at a ratio of 0.6 (w/w). (b) Calculated difference spectra of H5–Br-poly(dG-dC) at a ratio of 0.5 and H1–Br-poly(dG-dC) at a ratio of 0.6.

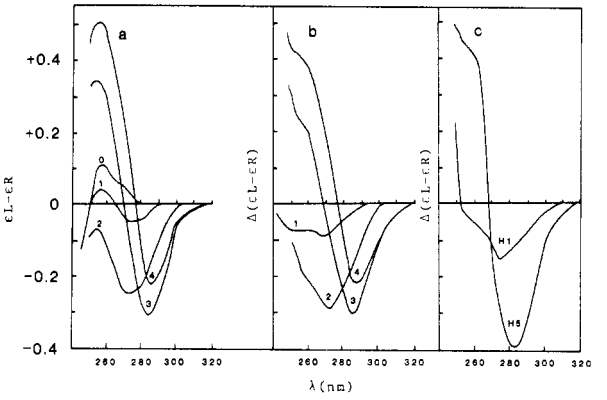

FIGURE 5: CD spectra of poly(dA-dT) and protein–poly(dA-dT) and calculated difference spectra. The solvent was 5 mM Tris–10 mM EDTA–150 mM NaCl, pH 7.6. (a) CD spectra as a function of H5–polynucleotide ratio of 0.1 (line 1), 0.2 (line 2), 0.4 (line 3), and 0.5 (line 4); poly(dA-dT), line 0. (b) Calculated difference spectra as a function of H5:polynucleotide ratio: 0.1 (line 1); 0.2 (line 2); 0.4 (line 3); 0.5 (line 4). (c) Calculated difference spectra of H5–poly(dA-dT) at a ratio of 0.5 and H1–poly(dA-dT) at a ratio of 0.6 (w/w).

nucleotide ratio of 0.6, the same qualitative changes were observed (Figure 3c) but they were less intense. Both the directly measured spectra and the calculated difference spectra demonstrated these changes to a deeply negative ψ spectrum form. Because the poly(dG-dC) was near the B–Z transition in the 2.0 M NaCl solution in which the initial mixture was made, experiments were also performed in which the polynucleotide and histone were mixed in 150 mM NaCl; again a deeply negative ψ spectrum was obtained.

Mixed with poly(dG-dC) in 2.0 M NaCl and dialyzed to lower ionic strength, core histones decreased the positive 270-nm peak to about 50% of that of the polynucleotide alone, a change similar to that observed by Prevelige & Fasman (1983). Lysozyme caused a slight increase in the positive 270-nm band and decreased the negative 249-nm band (data not shown).

A broad negative spectrum of much lower intensity was observed with H1 and H5 histone complexes of the Z-form, Br-poly(dG-dC) (Figure 4). The difference spectra were more interesting. They showed that H5 caused an increase in ellipticity above 290 nm and a sharp decrease below 285 nm. This gave the appearance of restoring B-like features to the spectrum, although at higher wavelengths than those characteristic for the right-handed poly(dG-dC) (Figure 4). Core histones caused a slight decrease in the intensity of the negative 290-nm ellipticity and lysozyme had no significant effect on the spectrum (not shown).

When complexes with poly(dA-dT) were prepared by gradient dialysis and then brought to 150 mM NaCl, the CD spectra changed in two stages as increasing amounts of histone H5 were added to the polynucleotide. At a low protein:polynucleotide ratio (0.1 or 0.2 w/w), a new negative band appeared with a maximal value at 275–280 nm and the positive band at 258 nm decreased (Figure 5). At higher protein:polynucleotide ratios (0.4 or 0.5), the new negative band increased in intensity and shifted to 285–288 nm, while the 255-nm band became more strongly positive (Figure 5a). The combined effect was to make the CD spectrum of the complex resemble an inverted form relative to that of B-DNA (Figure 5a). The two stages were clearly evident in difference spectra (Figure 5b).

When H1 was complexed with poly(dA-dT) at a ratio of 0.6, a negative band appeared, also centered at 277 nm, along with a positive peak at 258 nm. These changes were qualitatively similar to those of the first stage of change by H5, but the second stage was not observed (Figure 5c). Complexes prepared with core histones and brought to 150 mM NaCl showed only a decrease in the positive band at 258 nm (not shown).

Both high and low molecular weight poly(dA-dT) fractions were affected in similar ways on complexing with H5, but the precise conformational change in poly(dA-dT) did depend on polynucleotide size. Complexes made with larger molecules (800–1700 bp) displayed a slightly deeper minimum at a slightly lower wavelength in comparison with complexes containing smaller molecules of 100–350 bp.

When H5 was mixed with poly(dA-dT) in 150 mM NaCl rather than in high ionic strength followed by gradient dialysis, it caused appearance of a deeply negative ψ spectrum rather than the complex spectra shown in Figure 5. Lysozyme did not cause significant changes in the CD spectrum of poly(dA-dT) when it was mixed with the polynucleotide under the same conditions of gradient dialysis.

The effect of ionic strength was determined by addition of measured volumes of a stock 6 M NaCl solution to the complexes prepared by gradient dialysis. Changes in ionic strength affected the degree of negative ellipticity at a maximum of 288 nm for poly(dA-dT) complexes, 263 nm for poly(dG-dC) complexes, and 278 nm for poly[Br(dG-dC)] complexes (Figure 6). For all three polymers, the spectra were more dramatically changed by H5 than by H1 over a wide range of ionic strengths, with a maximal spectral change occurring between 0.1 and 0.2 M NaCl. The relatively small changes caused by lysozyme at these wavelengths are also indicated in Figure 6.

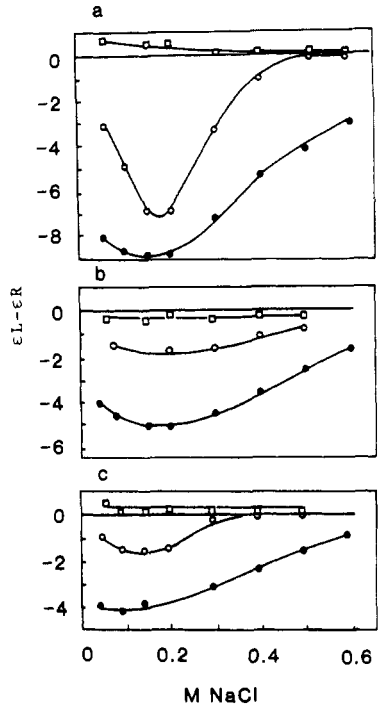

FIGURE 6: Effect of ionic strength on CD spectra for complexes of protein–polynucleotides. (a) Plot of the CD parameter ($\epsilon L - \epsilon R$) at 263 nm vs. the NaCl concentration for H5–poly(dG-dC) (●), H1–poly(dG-dC) (○), or lysozyme–poly(dG-dC) (□) complexes. (b) Plot of the CD parameter ($\epsilon L - \epsilon R$) at 278 nm vs. the NaCl concentration for H5–Br-poly(dG-dC) (●), H1–Br-poly(dG-dC) (○), or lysozyme–Br-poly(dG-dC) (□) complexes. (c) Plot of the CD parameter ($\epsilon L - \epsilon R$) at 288 nm vs. the NaCl concentration for H5–poly(dA-dT) (●), H1–poly(dA-dT) (○), or lysozyme–poly(dA-dT) (□) complexes.

Discussion

Our results indicate that Z-DNA and two forms of B-DNA differ in certain aspects in their interaction with H1 and H5 histones. By focusing on polynucleotides of regular base sequence but differing conformations, these experiments amplify previous observations of selectivity in reactions of lysine-rich histones with DNA. Previous work has established that H1 histones differ in reactions with (A+T)-rich and (G+C)-rich DNA (Šponar & Šormová, 1972; Hwan et al., 1975), that subfractions of H1 histones differ in their reactions with a given DNA (Welch & Cole, 1979; Corbett et al., 1980), and that H1 histone binds more strongly to supercoiled than to nicked circular or linear duplex DNA (Vogel & Singer, 1976). These indications of selectivity may be important in the functioning of these histones to condense certain regions of chromatin, thereby affecting the potential for gene expression in these regions.

Defined synthetic polynucleotides have been used to study nucleosome formation with core histones (Simpson & Kunzler, 1979; Bryan et al., 1979; Brahms et al., 1979; Prevelige & Fasman, 1983). Poly(dA-dT) and poly(dG-dC) differed in their associations with core histones (Prevelige & Fasman,

1983), and there were differences between their reconstituted nucleosomes and H5-stripped chick nucleosomes isolated from erythrocytes. Z-DNA did not form nucleosomes on mixture with core histones alone (Nickol et al., 1982) but did so under the influence of additional assembly protein (Miller et al., 1983). These findings again indicate potential for variability in organization of chromatin structure in regions of highly biased base sequence that may affect local DNA conformation.

In contrast with the core histones, which must form dimer or larger complexes in order to interact with DNA as they do in chromatin, lysine-rich histones bind to the linker DNA region separately. Although they make contact with H2a in the core particle (Boulikas et al., 1980), NMR studies and electron spin-label studies have shown that the interaction of H1 with free DNA is similar to its interaction with DNA in chromatin (Bradbury et al., 1975; Girardet & Lawrence, 1983). Measurements of variations in polynucleotide binding by lysine-rich histones, therefore, may reflect the potential for variable conformational changes within chromatin also.

In the interactions we observed, H5 caused larger effects than H1, in both the extent of polynucleotide binding and the extent of CD change. This may reflect the adaptation of H5 for formation of highly condensed inactive chromatin. In both H1 and H5, a central globular domain is flanked by more highly charged amino- and carboxyl-terminal domains (Allan et al., 1980). The globular region abuts the nucleosomal core particle near the entry–exit site, whereas the highly charged arms interact with the linker DNA (Thoma et al., 1983; Frado et al., 1983). The greatest sequence differences between the two histones occur in these charged domains, in which a higher arginine content occurs in H5. These differences may both increase the affinity of the histone–DNA interaction and modify the precise conformational changes caused by the binding; H5 was able to cause a CD spectral change in poly(dA-dT) that was not seen with H1. The conformational changes also depend on factors that determine how the DNA–histone association forms; simple mixing of poly(dA–dT) with H5 histone led to a ψ spectrum, whereas mixing at high ionic strength and gradient dialysis led to a more distinctive structure and spectrum.

The ψ spectrum, which was observed with poly(dG-dC) under any conditions of mixing, has also been observed when B-DNA of mixed base composition was placed in solution with organic polymers (Jordan et al., 1972), poly(L-lysine) (Shapiro et al., 1969), certain salts (Baase & Johnson, 1979), or H1 histone (Fasman et al., 1970; Olins & Olins, 1971). It may reflect side-by-side packing of DNA molecules with a long-range twisting order (Maestre & Reich, 1980). Such alignments could fit with the role of histones in condensing the simplest chromatin fibers into higher order structures (Thoma et al., 1983) and with the greater effectiveness of H5 over H1 in causing this condensation (Thomas & Rees, 1983). The difference spectrum between chicken erythrocyte nucleosomes that contain or lack lysine-rich histones is also a negative -band (Cowman & Fasman, 1980), indicating the role of this type of condensation in chromatin.

Other modes of condensation seem to be determined by the alternating (dA-dT) structure, especially in combination with H5. The H5–poly(dA-dT) spectrum we observed was similar to that formed on its combination with poly(L-lysine) (Shapiro et al., 1969) and to that which occurs in 3.4–3.6 M CsF (Vorlickova et al., 1983), except that in these two cases the rise from a negative band (at 275 nm) did not reach zero or form a positive CD peak at lower wavelengths. On the other hand, the 2.5 M NaCl spectrum of poly(dN2A-dT), in which a 2-amino group in adenine may favor transition to a left-handed conformation, did have a spectrum closer to what we observed with H5–poly(dA-dT) (Jovin et al., 1983). The polynucleotide containing 2-aminoadenine reacted weakly with anti-Z-DNA antibodies (Jovin et al., 1983); our H5–poly-(dA-dT) complexes did not (data not shown). The spectral change cannot be interpreted as sure evidence of the formation of Z-DNA (Tomasz et al., 1983). Poly(dA-dT) has not been shown to be converted to Z-DNA even though its alternating purine–pyrimidine sequence meets one of the requirements for that conversion. If interaction with H5 could interfere with the B-DNA-stabilizing water structure in the minor groove (Drew & Dickerson, 1981) of poly(dA-dT), perhaps it could favor the conversion of the polymer to a left-handed form (not precisely Z-DNA), as does the presence of the 2-amino group in poly(dN2A-dT) (Jovin et al., 1983). This would be opposite to the effect of the lysine-rich histones on poly(dG-dC), in which they have been found to stabilize the B-DNA under conditions near the B–Z transition (Russel et al., 1983). Analysis of difference spectra comparing H5–Br-poly(dG-dC) with the Z-DNA alone also suggested that combination with the histone reduced the Z-DNA features of the CD spectrum. This may have reflected a new distinct conformation, as the difference spectrum was not that of the pure B form of poly(dG-dC) (Figure 4).

Although complexes of histones and DNA cannot be considered as models of chromatin, these results suggest that different types of interactions between H1 and H5 histones and linker DNA may occur within chromatin. Such variations may modulate the structure in accordance with ionic changes, changes in the protein:DNA ratio, and the influence of other proteins, but some limitations or tendencies appear to be inherent in the DNA sequence and in the histones themselves. These properties may in turn determine the potential for maintaining functionally important chromatin domains in an active or inactive state.

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Registry No. Poly(dG-dC), 36786-90-0; poly(dA-dT), 26966-61-0.

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