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Conformational effects of histones H1 on DNA structure

Comparative study between H1-1, H1°, H5 and sperm holothuria ϕ_o

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Interactions of mammalian histones, H1-1 and H1°, ϕ_o from holothuria sperm and H5 with poly(dA-dT), poly(dG-dC) and poly(dG-me$_5$dC) were measured by a nitrocellulose filter binding assay and circular dichroism. All of the proteins bound to every one of the polymers, but differed in the extent of binding, which depended on the polynucleotide/protein ratios and ionic strength. The order of retention of all polymers was ϕ_o > H1-1 > H1°. The binding of H1° to poly(dG-me$_5$dC) was remarkably sensitive to ionic strength. The proteins caused changes in the spectral features of the polynucleotides, but differed in the type and extent of the change. Complexes prepared with H1-1 and H1° with all polymers showed a strongly negative ψ spectrum. Complexes of poly(dA-dT) and ϕ_o, at a protein/polynucleotide ratio of 0.4, displayed a distinctive spectrum, giving the appearance of a Z-like DNA spectrum, at low ionic strength. At higher ionic strength the complexes showed a ψ spectrum. Complexes of poly(dG-me$_5$dC) in the Z or B conformation with ϕ_o showed spectral features characteristic of a mixture of a Z-like and a ψ spectrum. In contrast, H5 reduced the Z-DNA spectral features in the presence of Mg, and produced an inversion of the B spectrum up to a polynucleotide/protein ratio of 0.24. These findings demonstrate the ability of different proteins to produce changes in the conformation of DNA. This may reflect the ability of chromatin to undergo differential condensation, depending on both the base composition of DNA and the type of H1 histone bound to it.

1. Introduction

The lysine-rich histones have been implicated in the formation and maintenance of high-order structure in chromatin. This family of proteins includes H1 and the histone H5, specific for nucleated erythrocytes, H1°, an analogous protein which is involved in the terminal differentiation of various cell types [1], and H1t, characteristic of rat testis chromatin (reviewed in ref. 2).

Changes in the expression of the genes coding for lysine-rich histones have been demonstrated during sea urchin development [3], spermatogenesis [4] and avian erythropoiesis [5], indicating that changes in the ratios of these histones may produce local and subtle changes in chromatin structure which may be important for regulating gene expression.

Association of the various H1 fractions with either native chromatin or H1-depleted chromatin, after reconstitution, has been extensively studied

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[6–10] showing that they may interact differently with DNA. They would have different effects on the process of condensation of chromatin to higher order structures, giving rise to regions of different stabilities. These differences may involve recognition of local variations in DNA composition and conformation. Studies with synthetic polynucleotides have indeed demonstrated that DNA presents structural differences between right-handed A-DNA, B-DNA, and left-handed Z-DNA. These structural differences may play a significant role in utilization of genetic information providing recognition sites for protein-DNA interaction (reviewed in ref. 11).

Studies on the conformation of the lysine-rich histones in solution have revealed three domains of which only the central one is folded. It is believed that the globular domain is responsible for the H1 location in chromatin, while the flanking domains give rise to the condensation of the DNA [12,13]. We have shown that calf H1 and one of the variants, H5, differ in binding and in changes in the CD spectra of polynucleotides of the B and Z conformation [14].

In order to gain further insights into the contributions made by the different domains of these proteins to their interaction with DNA, we have extended that study by using H1-1, H1° and ϕ_o. H1° has been considered to be a hybrid molecule, since it presents structural similarities with the globular and C-terminal domains of H5, and with the C-terminal region of H1 [15,16]. ϕ_o is a protein which occurs in the sperm of sea cucumber (*Holothuria tubulosa*). These cells contain the five somatic histones plus a unique basic protein ϕ_o, and a sperm-specific H1. ϕ_o is highly basic and represents 4% of the total weight of histones. It is only 78 amino acids long and its sequence is similar to the C-terminal region of H1 if arginine is considered equivalent to lysine [17].

In this study, we describe the interaction of the proteins with DNA of varying base composition (poly(dA-dT), poly(dG-dC), poly(dG-me_5dC)) and different conformation, since the methylated polymer exists in the B and Z conformation. We show that all the proteins bound to these polymers, but exerted different conformational effects on them, depending on the protein/polynucleotide ratio, as well as the ionic strength. These results are discussed in terms of models for chromatin physiology.

2. Materials and methods

2.1. Materials

Poly(dA-dT), poly(dG-dC) and poly(dG-me_5dC) were purchased from PL Biochemicals. They had a size distribution from 0.1 to 2.5 kb. The reagents used for nick translation were purchased from Amersham, except the unlabeled nucleotides which were obtained from Boehringer Mannheim. The polynucleotides were radiolabeled using the nick-translation technique described by Rigby et al. [18]. Nitrocellulose paper (pore size 0.45 μm) was a product of Schleicher and Schüll. Histone H5 was prepared from erythrocytes of adult white leghorn chickens [5], histones H1-1 and H1° were isolated from ox liver nuclei [7], and the protein ϕ_o was prepared from spermatozoan nuclei of the sea cucumber *H. tubulosa* [17].

The purity of histones was analyzed by following the SDS gel electrophoretic procedure of Thomas and Kornberg [19]. Protein concentration was determined as described by Lowry et al. [20].

Complexes of proteins and polynucleotides were prepared by step gradient dialysis according to the method of Simpson and Künzler [21]: complexes were prepared from mixtures of protein and polynucleotides in a solution of high ionic strength (2.0 M NaCl) followed by step 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 from the absorbance at 260 nm using the following extinction coefficients for 0.1% solutions: poly(dG-dC) and methylated polymer, 18; poly(dA-dT), 17.

The protein/polynucleotide ratios (P/p) are expressed by weight.

2.2. Filter binding assay

The technique followed to monitor the interaction between DNA and proteins has been described previously [14]. For any assay, 50 ng ^{3}H-

labeled polynucleotide were complexed with the appropriate amount of protein (200 ng) for determination of the ionic strength dependence of binding. The input radioactivity for a given polynucleotide is determined via the simple deposition of the same amount of polynucleotide solution on a filter.

2.3. Circular dichroism

CD spectra were obtained in a Jobin-Yvon DC III spectropolarimeter, using quartz cuvettes of 1 cm path length. Samples were usually scanned between 320 and 235 nm, at room temperature; the concentration of the samples was routinely determined by absorbance measurement at 258 nm and was always lower than 1 for CD experiments.

3. Results

3.1. Nitrocellulose filter retention

This approach utilizes the properties of nitrocellulose filter which binds DNA-protein complexes but not free double-helical DNA. For any particular polynucleotide/protein complex, the binding depended on the protein/polynucleotide ratio (P/p) from 0.6 to 20 (w/w) in the present experiments as well as on ionic strength.

Fig. 1 illustrates the binding of H1°, H1-1 and ϕ_0 to poly(dA-dT) (a), poly(dG-dC) (b) and poly(dG-me$_5$dC) (c) at 150 mM NaCl as a function of P/p ratios. The order of retention in every case was ϕ_0 > H1-1 > H1°. At 30 mM NaCl the binding of a given protein was not significantly different for all polynucleotides, the same effect being observed at 150 mM NaCl, except for the binding of H1° to the methylated polymer. At 30 mM NaCl, it bound 50% of the polynucleotide, while at 150 mM NaCl, binding was only 2%.

The effect of ionic strength on the binding of complexes to a nitrocellulose filter is demonstrated in fig. 2.

The order of retention of poly(dA-dT) and poly(dG-dC) was as above. For poly(dG-me$_5$dC) the order was H5 > ϕ_0, H1-1 > H1°. The binding

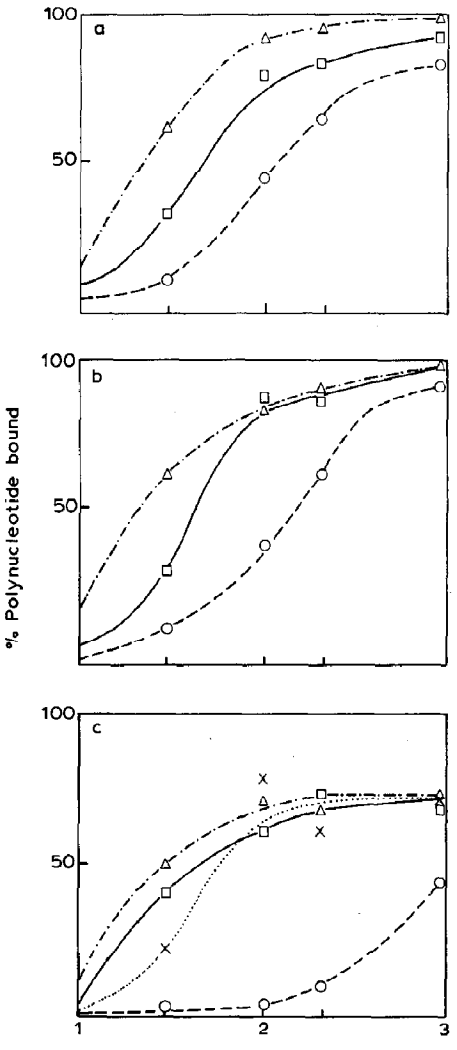

Fig. 1. Binding characteristics of histone H1° (○), H1-1 (□), H5 (×) and the protein ϕ_0 (△) to poly(dA-dT) (a), poly(dG-dC) (b) and poly(dG-me$_5$dC) (c). Increasing amounts of proteins were added to 50 ng ^{3}H-labeled polynucleotides in 5 mM Tris buffer (pH 7.6) containing 150 mM NaCl.

of H5 and H1° to the methylated polymer was quite different: H1° bound to the polymer at rather low ionic strength (less than 100 mM NaCl); conversely, H5 bound well above 100 mM NaCl, reaching 50% binding at 600 mM NaCl.

Such a hierarchy can be roughly estimated by calculating the ionic strength at which 50% of the polynucleotide was retained on the filter (table 1).

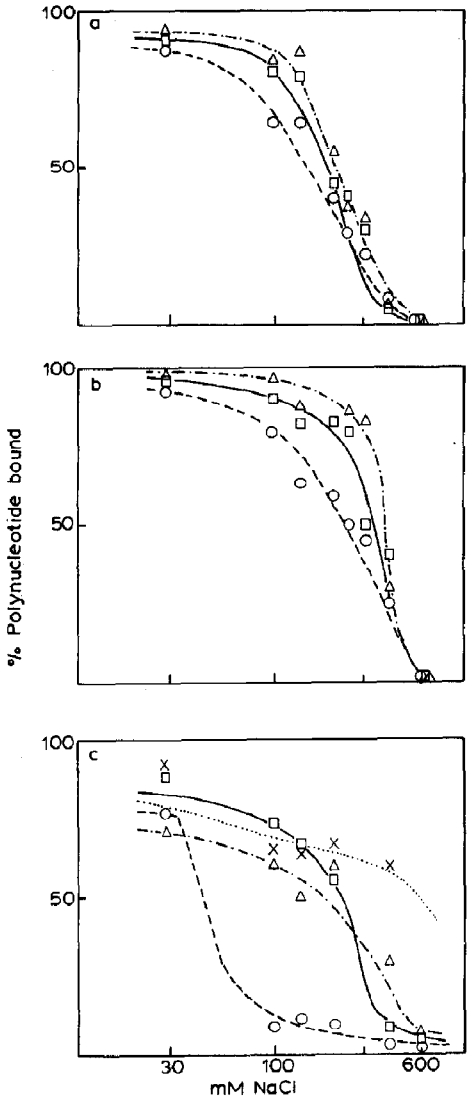

Fig. 2. Filter retention of protein-polynucleotide complexes at different ionic strengths. A mixture of 50 ng ^{3}H-labeled poly(dA-dT) (a), poly(dG-dC) (b) and poly(dG-me$_5$dC) (c) in 5 mM Tris (pH 7.6) at different NaCl concentrations was allowed to react with 200 ng H1° (○), H1-1 (□), H5 (×) and ϕ_o (△).

3.2. CD spectra

While filter retention study provides information about the stoichiometry of the polynucleotide-protein complexes, CD has been used to study the conformational changes of polynucleotides induced by this binding.

Table 1

NaCl concentration at which half-maximum binding was obtained for every protein-polynucleotide complex

	Poly(dA-dT)	Poly(dG-dC)	Poly(dG-medC)
H1-1	220 mM	340 mM	240 mM
H1°	200 mM	300 mM	70 mM
ϕ_o	250 mM	370 mM	270 mM
H5	300 mM [a]	340 mM [a]	> 400 mM

[a] Data from ref. 14.

Two different sets of experiments have been performed with polynucleotide-protein complexes; on the one hand, the complexes were prepared with a polynucleotide and a protein at various P/p ratios; then, the CD spectra were scanned for an ionic strength adjusted to 150 mM NaCl. On the other, samples were prepared at a given P/p ratio and CD spectra were scanned at different ionic strengths obtained by adding the appropriate amounts of 5 M NaCl.

3.3. Complexes obtained with H1-1 and H1°

With complexes obtained with H1-1 of H1°, at any P/p ratios and ionic strength, all three polynucleotides underwent the same qualitative transformation: the positive band at 270–275 nm disappeared and a new negative band appeared in the same wavelength range: 250–280 nm (fig. 3). This CD spectrum, termed the ψ spectrum, had already been observed when DNA of mixed composition was placed in solution with, for example, organic polymers [22] or H1 histone [23,24].

In the case of H1° complexes, for the same P/p ratios, the negative peak characteristic of a ψ spectrum was considerably deeper compared with those observed with H1-1 (fig. 3a and b). This was not observed with complexes obtained with the methylated polymer (fig. 3c). Furthermore, the presence of H1° gave rise to a ψ spectrum at lower ionic strengths than in the case of H1-1 (data not shown). No inversion of the B-spectrum was observed when H1-1 or H1° was complexed with any of the polymers used.

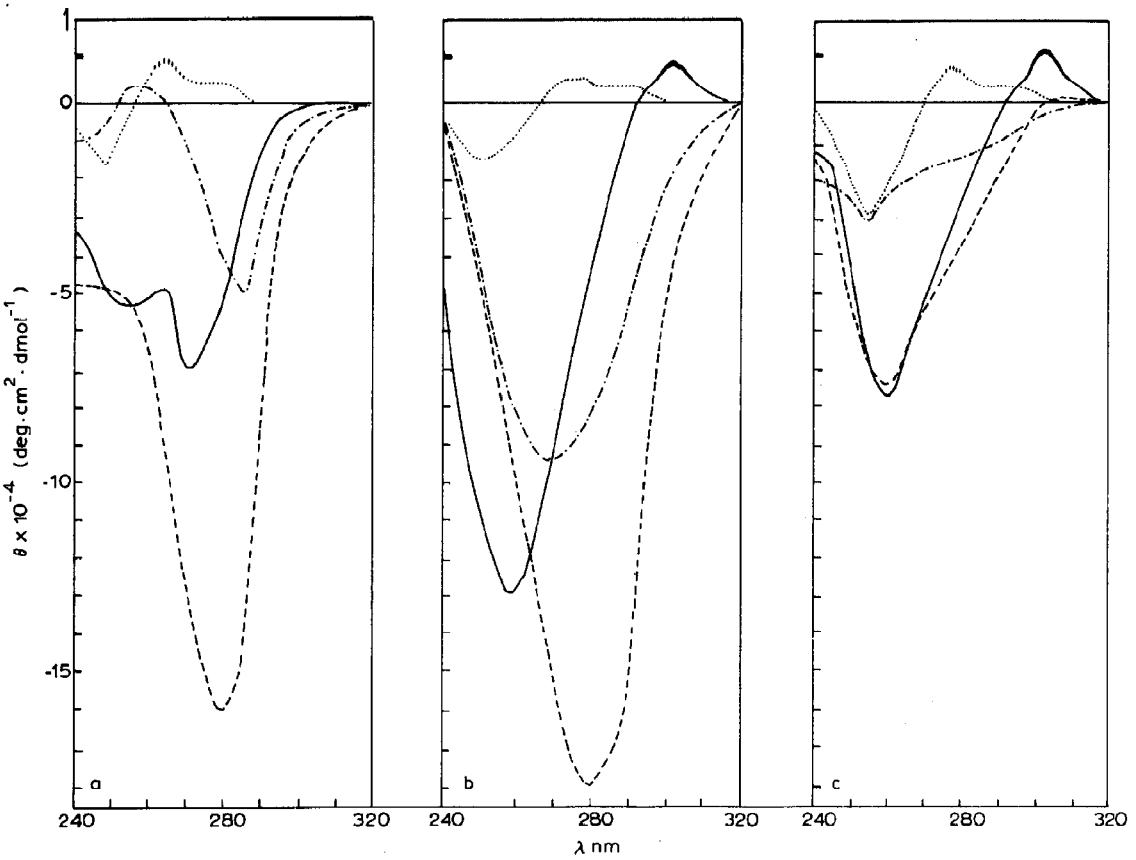

Fig. 3. CD spectra of protein-poly(dA-dT) (a), protein-poly(dG-dC) (b) and protein-poly(dG-me$_5$dC) (c) complexes. Solvent was 5 mM Tris, 10 mM EDTA, 150 mM NaCl (pH 7.6). The protein/polynucleotide ratio was 0.5 (w/w). (·····) Polynucleotide alone, (- - - - -) H1° complexes, (———) H1-1 complexes, (·–·–·) ϕ_o complexes.

3.4. Complexes obtained with ϕ_o

The effect of ϕ_o on the CD spectra of the various polynucleotides depended on the polymer used, the P/p ratio and on the ionic strength. When ϕ_o was complexed with poly(dG-dC) a ψ-type spectrum was obtained at any P/p ratio. Fig. 3b shows the CD spectrum for a P/p ratio of 0.5 at 150 mM NaCl. The same effect was observed at all ionic strengths studied at the same P/p ratio (data not shown).

When complexes with poly(dA-dT) were prepared and brought to 150 mM NaCl, the CD spectra underwent several stages of change as increasing amounts of ϕ_o were added. At a P/p ratio of 0.1, a negative band appeared with a maximal value at 276 nm and the positive peak at about 260 nm decreased. At a P/p ratio of 0.2–0.3, the negative band at 276 nm increased, and the band at 260 nm decreased even further. However at a P/p value of 0.4, the negative band decreased and shifted to 286 nm, while the positive band at 260 nm increased. The overall effect was to make this CD spectrum resemble an inverted form relative to that of B-DNA: we propose to call it a Z-like spectrum. At a P/p ratio of 0.5, the negative band attained values similar to that observed at a ratio of 0.4, while the positive band at 260 nm decreased below the value observed with the polynucleotide alone and shifted slightly to lower wavelength (fig. 4a).

When complexes with poly(dA-dT) were prepared at a P/p ratio of 0.5 and the spectra obtained at different ionic strengths, the overall

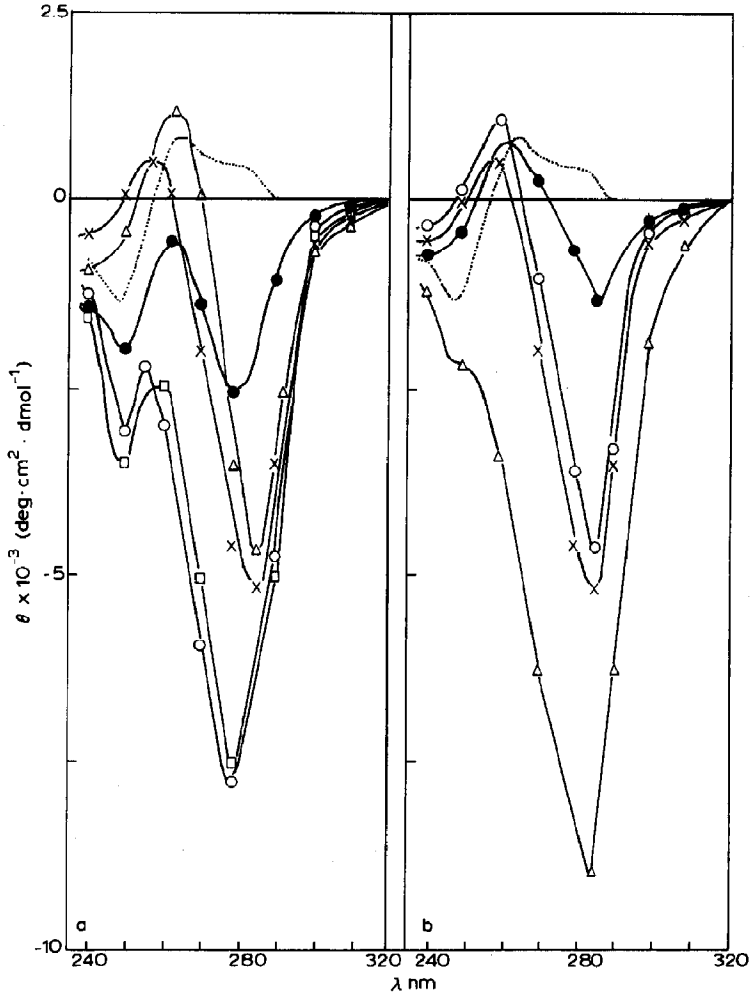

Fig. 4. CD spectra of poly(dA-dT)-ϕ_o complexes in 5 mM Tris, 10 mM EDTA (pH 7.6). (a) In 150 mM NaCl, increasing amounts of protein: $P/p = 0$ (·····), 0.1 (·–·–·), 0.2 (○———○), 0.3 (□———□), 0.4 (△———△), 0.5 (×———×). (b) Protein/polynucleotide ratio = 0.5, with increasing amounts of NaCl: (·–·–·) 20 mM, (○———○) 50 mM, (×———×) 150 mM, (△———△) 250 mM.

effect was similar to that described above. At 20 mM NaCl a negative band appeared at 287 nm and the positive band at 260 nm decreased slightly. Between 50 and 150 mM NaCl, the negative band increased markedly and the positive band increased slightly, giving rise to the Z-like spectra. However, between 250 and 400 mM NaCl, the negative band increased even further, shifting to 280 nm whereas the band at 260 nm decreased dramatically, the overall effect resembling the ψ spectra (fig. 4b).

When ϕ_o was complexed with the methylated polymer in the Z conformation, and the effect of the ionic strength studied, a mixture of Z-like and ψ spectra was observed. A negative shoulder was observed at 290 nm on a sharp negative band at 260 nm and a positive band at 240 nm (fig. 5a).

When complexes were obtained at different P/p ratios with the methylated polymer in the B conformation (fig. 6a), the spectra changed according to the P/p ratio: at $P/p = 0.24$, a negative band was observed at 290 nm with a

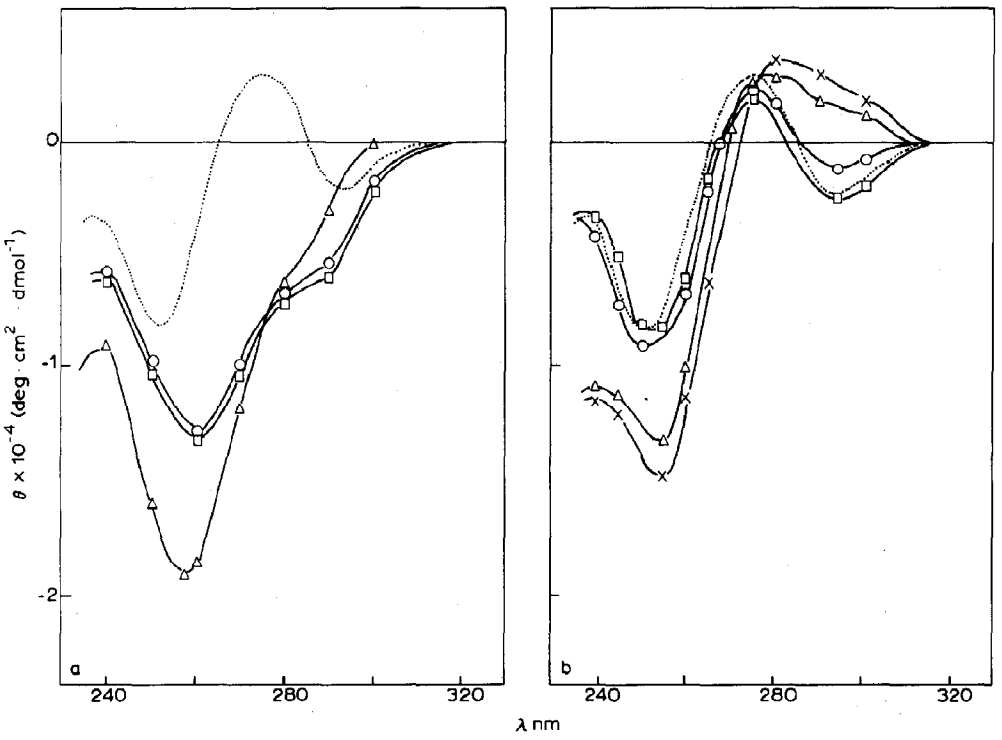

Fig. 5. CD spectra of protein-poly(dG-me$_5$dC) in 5 mM Tris, 2 mM $MgCl_2$ (pH 7.6) for a protein/polynucleotide ratio of $P/p = 0.4$, with increasing amounts of NaCl: (·····) polynucleotide alone, (○———○) 20 mM NaCl, (□———□) 100 mM NaCl, (△———△) 200 mM NaCl, (×———×) 300 mM NaCl. (a) ϕ_o-poly(dG-me$_5$dC), (b) H5-poly(dG-me$_5$dC).

positive band near 240 nm; at $P/p = 0.5$ a mixture of Z-like and ψ spectra was obtained as in the case of the polymer in the presence of Mg^{2+} (fig. 5a).

3.5. Complexes obtained with H5

The interaction of H5 with the methylated polymer was more complicated. When H5 was complexed with poly(dG-me$_5$dC) in the presence of 2 mM $MgCl_2$, at a P/p ratio of 0.4, the CD spectra underwent two stages of change as the ionic strength increased. At 20–100 mM NaCl, subtle changes were observed, a slight increase of the negative bands at 255 and 290 nm, with a slight shift of the former to greater wavelength. At higher ionic strengths, the negative band at 290 nm disappeared and the B-type features of the spectrum were restored (fig. 5b). This effect has been observed on complexes of H5 with Br-poly(dG-dC) [14].

When H5 was complexed with poly(dG-me$_5$dC) and brought to a final ionic strength of 150 mM NaCl, the spectra changed as increasing amounts of histone were added. Up to a P/p ratio of 0.3, a negative band appeared with a maximum at about 290 nm and a positive band with a maximum at 240 nm (fig. 6b). For a P/p ratio equal to 0.5, the CD spectrum is fully of the ψ type. When the effect of ionic strength was studied on a complex poly(dG-me$_5$dC) the ψ spectrum was observed for all ionic strengths studied (not shown).

Some of the complexes giving a ψ-type spectrum at higher protein and salt concentration were opalescent. After centrifugation (at 10000 × g for 30 min) the CD spectrum of the supernatants was of the B type and the absorbances at 260 nm were dramatically lower. However, the complexes

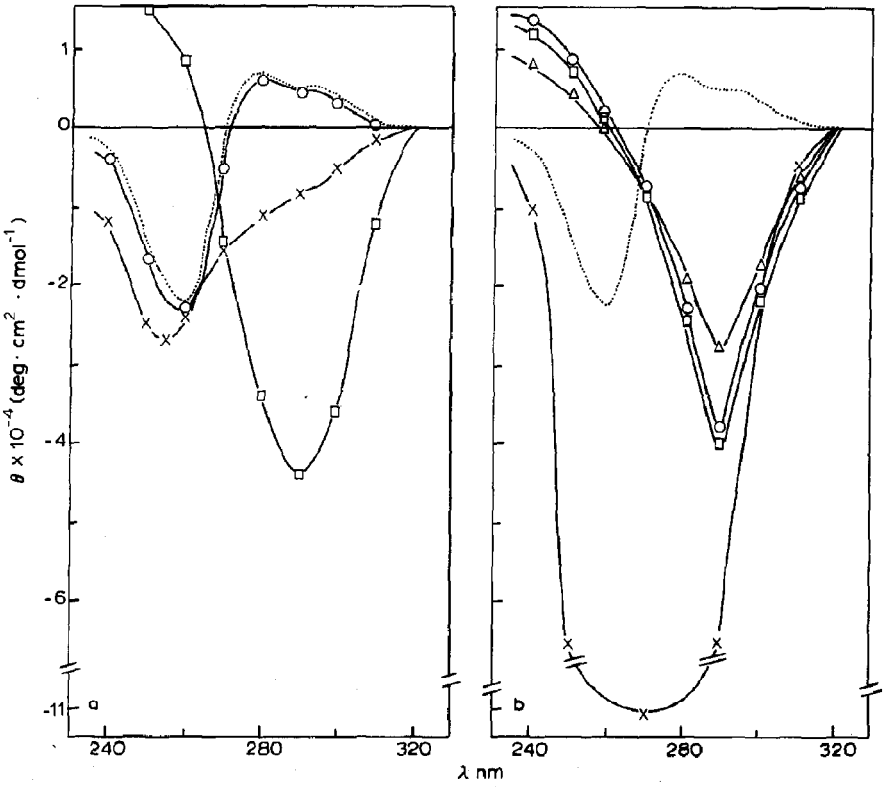

Fig. 6. CD spectra of protein-poly(dG-me$_5$dC) in 5 mM Tris, 10 mM EDTA, 150 mM NaCl (pH 7.2). (a) CD spectra as a function of ϕ_o/polynucleotide ratio. (b) CD spectra as a function of H5/polynucleotide ratio. (·····) Polynucleotide alone, (○——○) $P/p = 0.16$, (□——□) $P/p = 0.24$, (△——△) $P/p = 032$, (×——×) $P/p = 0.5$.

formed by methylated polymer in the presence of Mg^{2+} were transparent in all the cases studied.

A summary of the B-Z-ψ interconversion is given in fig. 7.

4. Discussion

We have previously shown that B- and Z-DNA differ in their interaction with H1 and one of its variants, H5 [14]. This indication of selectivity may be important in determining the role of these histones in chromatin condensation, since they interact primarily with DNA, rather than with the core histones.

We have expanded that study by comparing the effect of H1-1, H1°, H5 and the protein ϕ_o on filter retention of polynucleotides of defined sequences, as well as the effect of these proteins on the CD spectra. We observed that H5 and ϕ_o had a greater effect on polynucleotide binding as well as on the extent of CD change.

These proteins bound to all nucleotides studied in the following order ϕ_o > H1-1 > H1°. This order was the same when the P/p ratio remained constant for increasing ionic strengths. In the case of the methylated polymer, the difference in interaction was much more pronounced (table 1).

The conformational change leading to the Z-DNA form, first observed in poly(dG-dC), takes place in the presence of molar quantities of NaCl [11]. Another conformation of DNA, the ψ state, has been observed and interpreted by Maestre and Reich [25] as side-by-side packing of DNA molecules [23,26]. Several types of B → ψ transitions have been described, giving rise to either a ψ_+ or a

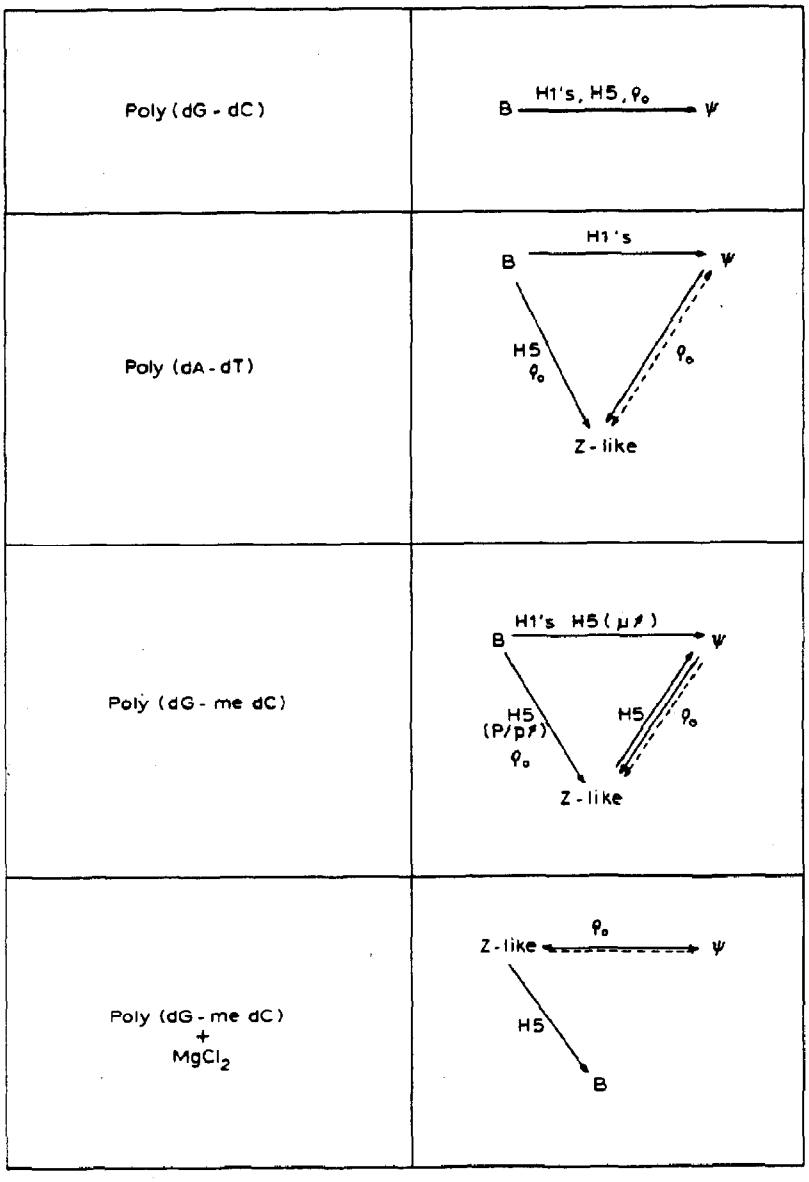

Fig. 7. Summary of B-Z-ψ interconversions. ($\rightarrow$) Change from conformation B to ψ, or B to Z or Z to ψ. ($\rightleftharpoons$) Mixture of spectra Z + ψ.

ψ_- spectra, involving various factors in the sign of the ellipticity [27–29].

In the present study, we have addressed the question of the potential role of lysine-rich histones in such conformational recognition and/or transition.

When complexes were obtained with H1-1 or H1°, a transition from the B to the ψ form appears as the P/p ratio or ionic strength increases. The latter observation was interpreted as the result of the C-terminal domain being responsible for condensation of the DNA [30]. A different

type of interaction was observed when ϕ_o was bound to the different nucleotides: the complex formed with poly (dG-dC) gave the ψ spectrum under all conditions studied. However, under specified conditions of ionic strength and P/p, the complexes formed with poly(dA-dT) and poly(dG-me_5dC) give a Z-like spectrum as an intermediate between the B and ψ types. It has been documented that A-T base-pairs form Z-DNA less readily than G-C, even though this polymer presents an alternating purine-pyrimidine sequence which would facilitate the transition. Inversion of the B spectrum to give a Z-like spectrum has been observed in 2.5 M NaCl for poly(dN_2A-dT), in which a modification of the 2-amino group in adenine may favor a left-handed transition. Recently, a Z spectrum of poly(dA-dT) has been obtained in the presence of nickel ions [31]. It is possible that H5 and ϕ_o interfere with the stabilizing water structure in the minor groove of poly(dA-dT) and favor the conversion to an inverted B spectrum [32]. These two proteins differ, however, in their interactions with the methylated polymer. ϕ_o induces what appears to be the result of a transition between Z-like and ψ spectra. Similar types of spectra have been obtained with complexes of lysine-rich histones and poly(dG-dC) and interpreted as being C-type spectra [33].

H5 induces the same B → Z-like → ψ transition with increasing P/p ratio; however, only the ψ form was obtained directly when the ionic strength increased. When H5 interacts with the polymer in the Z conformation, it reduces the features of the Z form and stabilizes the B conformation. This resembles the interaction of H5 with another Z-DNA polymer, Br-poly(dG-dC) [14]. This result also agrees with the observation that H5 induces the B conformation upon mixing with poly(dG-dC) in the Z form [33].

The final conformation in the present study is always ψ_-, in agreement with the result obtained on polylysine [34], which could be representative of a less compact quaternary structure. On the other hand, an intermediate step is only observed when poly(dA-dT) and poly(dG-me_5dC) are complexed with H5 or ϕ_o: then a B → Z-like → ψ-transition occurs.

In all lysine-rich histones, a central globular domain is flanked by a more strongly charged amino and carboxyl-terminal half [12]. The greater sequence difference between these histones occurs in these charged domains in which a higher arginine content occurs in H5. H1° presents structural homologies with H5 in the central globular domain as well as in the C-terminal half. As it also shows structural homologies with H1, it is considered to be a hybrid protein. Conversely, ϕ_o is highly basic and is only 78 amino acids in length. Its sequence is similar to the C-terminal half of calf thymus H-1 [17] and it can be a useful model of this part of the molecule.

In spite of the structural homologies between H1° and H5, the binding of H1° to all the nucleotides studied resembled that of H1. These variations cannot be explained simply by differences in charge, but rather in sequences and combinations of domains. One of the factors which may be important in the interactions of H1° and H5 with polynucleotides may be involved with the replacement of Ser-145 and Ser-166 in H5 by leucine and proline in H1° [35]. These two residues have been shown to be phosphorylated in H5 by a rat kinase and to be of importance for the protein-DNA interaction [36].

The fact that the conformational changes depend on factors that determine how the DNA-protein association forms indicates that the folded central region may be crucial in modulating the interaction of the C-terminal domain with DNA.

Other contributions to this modulation may derive from the N-terminal domain. Studies on sequence alignment have shown that while the globular region is the most conserved of the domains, more variations are expressed in the disordered N- and C-terminal region. The N-terminal domain of calf H1 contains conserved clusters of basic residues which may be important for DNA-protein interaction. Two out of three domains are also present in H5 [37].

The overall difference in interaction of ϕ_o and H1-type histones with polynucleotides of defined sequence indicates that the C-terminal domain of the lysine-rich histones could indeed be responsible for the condensation of DNA, with the N-terminal and globular domains acting as mod-

ulators of this interaction. This may have important biological implications, since the presence of H5 has been associated with inactivation of the avian genome, while that of H1° has been proposed to be connected with differentiated states and cells that are not replicating.

On the other hand, clusters of A-T are found in eukaryotic DNA, as well as cytosine methylation which has been associated with gene inactivity in vivo [38]. Furthermore, it has been speculated that methylation might have an effect on the ability of DNA to interact differently with histones so that the chromatin structure or stability is altered [39]. The differential interaction of the H1 proteins with these types of DNA may have a biological significance.

The combination of at least these three modulating factors, namely, base composition, chemical modification of DNA and histone primary structure, may be involved in the high-range regulation of gene expression in eukaryotic cells.

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Addendum

During the preparation of the present paper, Ausio and co-workers [40] published a report concluding on the absence of any B → Z-DNA transition when core particles reconstituted with poly(dG-me_5dC) are subjected to $MgCl_2$ addition. There is no contradiction with our experiments, which are performed with long-size polynucleotides and H1 family histones.

References

1 R. Gjerset, C. Gorka, S. Hasthorpe, J.-J. Lawrence and H.W. Eisen, Proc. Natl. Acad. Sci. U.S.A. 79 (1982) 2333.
2 R.S. Wu, H.T. Danusz, C.L. Hatch and W.M. Bonner, CRC Crit. Rev. Biochem. 20 (1986) 201.
3 K.M. Newrock, C.R. Alfageme, R.V. Nardi and L.H. Cohen, Cold Spring Harbor Symp. Quant. Biol. 42 (1977) 421.
4 S.M. Seyedin and S.W. Kisjler, Biochemistry 18 (1979) 1371.
5 C.V. Mura, P.C. Huang and D.A. Levy, J. Immunol. 113 (1974) 750.
6 C. Gorka and J.-J. Lawrence, Nucleic Acids Res. 7 (1979) 347.
7 J. Biard-Roche, C. Gorka and J.-J. Lawrence, EMBO J. 1 (1982) 1487.
8 J. Roche, C. Marion, C. Gorka, B. Roux and J.-J. Lawrence, Biochem. Biophys. Res. Commun. 121 (1984) 530.
9 C. Marion, J. Roche, B. Roux and C. Gorka, Biochemistry 24 (1985) 6328.
10 J. Roche, J.-L. Girardet, C. Gorka and J.-J. Lawrence, Nucleic Acids Res. 13 (1985) 2843.
11 A. Rich, A. Nordheim and A.H.J. Wang, Annu. Rev. Biochem. 53 (1984) 791.
12 I. Allan, P.G. Hartman, C. Crane-Robinson and F.X. Aviles, Nature 288 (1980) 675.
13 J.-L. Girardet and J.-J. Lawrence, Eur. J. Biochem. 132 (1983) 195.
14 C.V. Mura and B.D. Stollar, Biochemistry 23 (1984) 6147.
15 C.V. Mura and B.D. Stollar, J. Biol. Chem. 256 (1981) 9767.
16 B.A. Neary, C.V. Mura and B.D. Stollar, J. Biol. Chem. 260 (1985) 15850.
17 A. Jordan, Ph.D. Thesis, Faculty of Chemistry, University of Barcelona (1982).
18 P.W.J. Rigby, M. Dieckmann, C. Rhodes and P. Berg, J. Mol. Biol. 113 (1977) 237.
19 J.O. Thomas and R.D. Kornberg, Proc. Natl. Acad. Sci. U.S.A. 72 (1975) 2626.
20 O.H. Lowry, N.J. Rosebrough, A.L. Farr and R.J. Randall, J. Biol. Chem. 193 (1951) 265.
21 R.T. Simpson and P. Kunzler, Nucleic Acids Res. 6 (1979) 1387.
22 C.F. Jordan, L.S. Lerman and J.H. Venable, Nat. New Biol. 236 (1972) 67.
23 G.D. Fasman, B. Schaffhausen, L. Goldsmith and A. Adler, Biochemistry 9 (1970) 2814.
24 D.E. Olins and A.L. Olins, J. Mol. Biol. 57 (1971) 437.
25 M.F. Maestre and C. Reich, Biochemistry 19 (1980) 5214.
26 J. Sponar and I. Fric, Biopolymers 11 (1972) 2317.
27 S. Stokrova, J. Sponar, M. Havranek, B. Sedlacek and K. Blaha, Biopolymers 14 (1975) 1231.
28 Y.A. Shin and G.L. Eichhorn, Biopolymers 16 (1977) 225.
29 Y.A. Shin, S.L. Feroli and G.L. Eichhorn, Biopolymers 25 (1986) 2133.
30 F. Moran, F. Montero, F. Azorin and P. Suau, Biophys. Chem. 22 (1985) 125.
31 S. Adam, J. Liquier, J.A. Taboury and E. Taillandier, Biochemistry 25 (1986) 3220.
32 A.H.J. Wang, T. Hakoshima, G. van der Marel, J. van Boom and A. Rich, Cell 37 (1984) 321.

33 E. Russo, V. Giancotti and C. Crane-Robinson, Int. J. Biol. Macromol. 5 (1983) 366.
34 Y.A. Shin and G.L. Eichhorn, Biopolymers 23 (1984) 325.
35 D. Doenecke and R. Tonjes, J. Mol. Biol. 187 (1986) 461.
36 G. Briand, D. Kniecik, P. Sautiere, D. Wouters, O. Borie-Loy, G. Biserte, A. Mazen and M. Champagne, FEBS Lett. 112 (1980) 147.
37 L. Bohm and T.C. Mitchell, FEBS Lett. 193 (1985) 1.
38 W. Doerfler, Annu. Rev. Biochem. 52 (1983) 93.
39 G. Felsenfeld, J. Nickol, M. Behe, J. McGhee and D. Jackson, Cold Spring Harbor Symp. Quant. Biol. 47 (1982) 577.
40 J. Ausio, G. Zhou and K. van Holde, Biochemistry 26 (1987) 5595.