Chen, S. C., Sturtevant, J. M., & Gaffney, B. J. (1980) *Proc. Natl. Acad. Sci. U.S.A. 77*, 5060–5063.
Comfurius, P., & Zwaal, R. F. A. (1977) *Biochim. Biophys. Acta 488*, 36–42.
Cullis, P. R., & De Kruijff, B. (1979) *Biochim. Biophys. Acta 599*, 399–420.
Elder, M., Hitchcock, P., Mason, R., & Shipley, G. G. (1977) *Proc. R. Soc. London, Ser. A 354*, 157–170.
Finean, J. B. (1953) *Biochim. Biophys. Acta 10*, 371–384.
Finean, J. B., & Millington, P. F. (1955) *Trans. Faraday Soc. 51*, 1008–1015.
Füldner, H. H. (1981) *Biochemistry 20*, 5707–5710.
Harlos, K., & Eibl, H. (1980) *Biochim. Biophys. Acta 601*, 113–122.
Harlos, K., & Eibl, H. (1981) *Biochemistry 20*, 2888–2892.
Hauser, H., Pascher, I., Pearson, R. H., & Sundell, S. (1981) *Biochim. Biophys. Acta 650*, 21–51.
Hitchcock, P. B., Mason, R., Thomas, K. M., & Shipley, G. G. (1974) *Proc. Natl. Acad. Sci. U.S.A. 71*, 3036–3040.
Hui, S. W., & Boni, L. T. (1982) *Nature (London) 296*, 175–176.
Inoko, Y., & Mitsui, T. (1978) *J. Phys. Soc. Jpn. 44*, 1918–1924.
Janiak, M. J., Small, D. M., & Shipley, G. G. (1976) *Biochemistry 15*, 4575–4580.
Janiak, M. J., Small, D. M., & Shipley, G. G. (1979) *J. Biol. Chem. 254*, 6068–6078.
Levine, Y. K., & Wilkins, M. H. F. (1971) *Nature (London) 230*, 69–72.
Lis, L. J., McAlister, M., Fuller, N., Rand, R. P., & Parsegian, V. A. (1982) *Biophys. J. 37*, 657–666.
Luzzati, V. (1968) in *Biological Membranes* (Chapman, D., Ed.) Vol. 1, pp 71–123, Academic Press, London.
Luzzati, V., Gulik-Krzywicki, T., & Tardieu, A. (1968) *Nature (London) 218*, 1031–1034.
Mantsch, H. H., Hsi, S. C., Butler, K. W., & Cameron, D. G. (1983) *Biochim. Biophys. Acta 728*, 325–330.
McIntosh, T. J. (1980) *Biophys. J. 29*, 237–246.
Nagle, J. F., & Wilkinson, D. A. (1978) *Biophys. J. 23*, 159–175.
Rand, R. P., Tinker, D. O., & Fast, P. G. (1971) *Chem. Phys. Lipids 6*, 333–342.
Reiss-Husson, F. (1967) *J. Mol. Biol. 25*, 363–382.
Rilfors, L., Khan, A., Brentel, I., Wieslander, A., & Lindblom, G. (1982) *FEBS Lett. 149*, 293–298.
Ruocco, M. J., & Shipley, G. G. (1982a) *Biochim. Biophys. Acta 684*, 59–66.
Ruocco, M. J., & Shipley, G. G. (1982b) *Biochim. Biophys. Acta 691*, 309–320.
Seddon, J. M. (1980) Ph.D. Thesis, University of London.
Seddon, J. M., Cevc, G., & Marsh, D. (1983a) *Biochemistry 22*, 1280–1289.
Seddon, J. M., Harlos, K., & Marsh, D. (1983b) *J. Biol. Chem. 258*, 3850–3854.
Shipley, G. G. (1973) in *Biological Membranes* (Chapman, D., & Wallach, D. F. H., Eds.) Vol. 2, pp 1–89, Academic Press, London.
Small, D. M. (1967) *J. Lipid Res. 8*, 551–557.
Tardieu, A., Luzzati, V., & Reman, F. C. (1973) *J. Mol. Biol. 75*, 711–733.
Wilkinson, D. A., & Nagle, J. F. (1981) *Biochemistry 20*, 187–192.

Role of the Protein α Helixes in Histone–DNA Interactions Studied by Vibrational Spectroscopy†

E. Taillandier,* L. Fort, J. Liquier, M. Couppez, and P. Sautiere

ABSTRACT: The localization of structurally important histone–DNA interactions has been investigated by vibrational spectroscopy. Histones H2A, H2B, and H4 and their fragments (H2A, 1–56, 1–89, 73–129; H2B, 1–59, 1–83; H4, 1–53, 1–67, 1–84, 69–84, 85–102) have been prepared, characterized, and used to reconstitute protein–DNA complexes. Evidence is given for the existence of a direct relationship between the presence of ordered α-helical structures in the histones and a stabilization of the DNA in a B geometry. Infrared linear and ultraviolet dichroism measurements indicate that the N-terminal fragments, rich in basic residues and mostly in a random conformation, remain without any influence on the secondary structure of the nucleic acid, leaving it free in the complexes to undergo a total B → A conformational transition. On the contrary, histone fragments that involve some α-helical parts of the protein partially stabilize the DNA in a B geometry. Histone fragments that contain all of the α helixes of the protein block the DNA in the same way as the whole corresponding histone. A model for histone–DNA interactions in the core particle is discussed.

The main features of chromatin structure have now been known for some years, and the repetitive unit of this nucleoprotein, the core particle, has been characterized with precision [for a review, see Felsenfeld (1978) and Weisbrod (1982)]. However, the exact location of the important DNA–histone interactions and particularly the involved histone residues is not known. Recent cross-linking experiments (Shick et al., 1980) have shown that in the core particle histones are bound to regularly arranged discrete DNA segments. Thus, starting from the 5′-end of the 144-bp core DNA, three main sites are obtained for H4 (around positions 45, 55, and 65) and likewise for the other core histones, each DNA fragment being about six nucleotides long. Earlier trypsin and nuclease digestion studies (Weintraub & Van Lente, 1974) are consistent with models in which the N terminal basic ends of histones do not belong to the protein core of the nucleosome. We have previously shown that vibrational spectroscopy is a very useful

† From the Laboratoire de Spectroscopie Biomolēculaire, Universite Paris-Nord, 93000 Bobigny, France (E.T., L.F., and J.L.), and the Unité associée CNRS No. 409, Institut de Recherches sur le Cancer 59045, Lille, France (P.S. and M.C.). *Received August 12, 1983; revised manuscript received December 28, 1983.* P.S. and M.C. are indebted to the Centre National de la Recherche Scientifique for Grant Action Thématique Programmée Chromatine 4203.

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technique to study the secondary structures of DNA free or in nucleoproteins, as well as the conformational transitions of the nucleic acid (Liquier et al., 1977; Taillandier et al., 1979). In these studies, the stabilization of the B geometry of the DNA by the core histones has been proved. Here, we investigate the localization of the interaction responsible for this loss of conformational flexibility of DNA, by studying nucleohistones reconstituted with well-defined H2A, H2B, and H4 fragments. A direct relationship between the presence of ordered secondary structure in the protein and its stabilizing effect on the DNA is found: the N-terminal fragments of H2A, H2B, and H4 remain quite inefficient with respect to the stabilization of the nucleic acid in a particular geometry, whereas the α helical parts of these histones block the DNA in a B geometry.

Materials and Methods

Preparation of Histone Fragments. Histones H2A, H2B, and H4 were extracted from calf thymus chromatin by selective extraction methods of Johns (1964, 1967) and purified as described by Michalski-Scrive et al. (1982).

The H4 fragments 1–84 and 85–102 and the H2B fragment 1–59 were obtained by cyanogen bromide cleavage of the histones according to the method of Gross & Witkop (1962). The H4 fragments 1–67 and 69–84 and the H2A fragments 1–89 and 73–129 were obtained by cleavage at aspartyl residues with 0.25 M acetic acid (10 mg of protein/mL) at 105 °C (Schroeder et al., 1963). V8 staphylococcal protease hydrolysis in 0.05 M ammonium acetate at pH 4.0 and 37 °C was used to prepare the fragments 1–53 from H4 (Couppez et al., 1980) and 1–56 from H2A. All these fragments were isolated and purified through gel filtration chromatography on Bio-Gel P-10 and/or Sephadex G-50 or G-100 equilibrated and eluted with 10 mM HCl saturated with chloroform (see paragraph at end of paper regarding supplementary material).

Limited chymotryptic hydrolysis of histone H2B (100 mg) was performed in 10 mL of 0.1 M ammonium bicarbonate–0.5 M NaCl at pH 8.5 and 20 °C for 5 h with an enzyme-to-substrate ratio of 1:5000 (w/w). The enzyme was inactivated by addition of solid guanidinium chloride up to a final concentration of 4 M and of 2-mercaptoethanol up to a final concentration of 0.3 M. The digest was left overnight at room temperature. It was then acidified to pH 2.0 and fractionated on two columns of Sephadex G-100 (90 × 5 cm) coupled in series, with 10 mM HCl saturated in chloroform as eluant.

The purity of the histone fragments was checked by polyacrylamide slab gel electrophoresis at pH 2.7 in 2.5 M urea (Panyim & Chalkley, 1969) at a 17% acrylamide concentration. The histone fragments were characterized by amino acid analysis performed on a Beckman 119 CL amino acid analyzer after hydrolysis in 6 M HCl (1 mL/mg of protein) at 110 °C for 24 h in vacuo, with one drop of 1% phenol to avoid excessive degradation of tyrosine.

Complex Preparation. Salmon sperm DNA (Worthington or Sigma) was dissolved in 1.5 M NaCl at a concentration of 5–7 mg/mL. Exact concentrations were determined by UV absorbance measurements under the assumption of $A_{260} = 20$ for a 1 mg/mL solution and a 1-cm path length. Histones were dissolved in 10^{-3} M HCl (concentration 1 mg/mL). An identical volume of 3 M NaCl was added to the solution immediately before mixing with the DNA. A stepwise dialysis was then performed at 4 °C against 1.2, 0.75 and 0.35 M NaCl (2 h each) and finally overnight dialysis against NaCl solutions between 10^{-2} and 5.10^{-3} M, determined to obtain a NaCl to DNA weight ratio of 6–9% in the sample (free DNA in such conditions undergoes a total B → A transition). The exact amount of Na^+ was measured by flame photometry. The DNA to protein ratios were controlled by infrared spectroscopy, and the histone content of the samples was checked by Lowry measurements (Lowry et al., 1951).

Circular Dichroism. CD spectra were obtained on a Jobin-Yvon dichrograph R. J. Mark III with a 0.1-mm pathlength cell. Measurements were performed at 4 °C, at a concentration of histone or histone fragment of 0.5–1 mg/mL in 10 mM sodium phosphate buffer, pH 6.5, in absence or in presence of 2 M NaCl. θ is expressed in deg cm^2 (dmol of residues)$^{-1}$. Protein or peptide concentrations were determined from the tyrosine absorption coefficient at 275 nm ($\epsilon \simeq 1350$ M^{-1} cm^{-1}) and from amino acid analysis for peptides devoid of tyrosine.

Infrared Spectroscopy. The infrared spectra were recorded on a Perkin-Elmer 180 double-beam ratio recorder spectrophotometer (between 4000 and 700 cm^{-1}) equiped with a wire grid polarizer (KRS 5 support) placed in the common beam and oriented at 45° with respect to the slits and the preferential stretching direction of the samples. The complexes were simply gently dried after being deposited on Irtran 2 windows and oriented by unidirectional mechanical stroking when polarized measurements were wished. Relative humidity monitoring of the sample cells was achieved as described previously (Liquier et al., 1977). The infrared data (wavenumber and ratio of sample and reference beam intensities) are directly transferred to a HP 9825 A computer associated to the spectrophotometer. The absorbance values of the bands are calculated by using as a base line the spectrum of the measurement cell stored in the computer, thus eliminating any possible differential contribution between sample and reference cells. The water contents of the hydrated films are determined by using the absorption below 750 cm^{-1}, where no important DNA or protein band is observed. The water spectrum is then recalculated, and scaled spectrum substraction is achieved, leaving only the DNA and protein absorptions. Several possibilities of data treatment such as determination of DNA conformations in mixtures of geometries, computation of dichroic ratios and orientation parameters, and characterization of protein absorptions in the nucleoproteic complexes were systematically used.

Results

Identification of Histone Fragments. The amino acid compositions of the different fragments used in this study are presented in Table I.

The hydrolysis of calf histone H2B with chymotrypsin at high ionic strength (0.5 M) and at a very low enzyme-to-substrate ratio (1:5000) reduced drastically the number of bonds susceptible to chymotryptic cleavage. Indeed, at this salt concentration, histone H2B has a maximum of secondary and tertiary structures, and most of the potential sites for chymotryptic cleavage, which are located in the organized regions of the molecule, have become inaccessible to the enzyme. Thus, the H2B fragment 1–83 was preferentially obtained and was separated from the uncleaved protein and from smaller fragments by chromatography on Sephadex G-100 (Figure 1). Fragment 1–83 was obtained in pure form in the fraction indicated by a solid bar. Due to the way in which it was obtained, fragment 1–83 was determined not only from amino acid analysis (Table I) but also from structural data provided by manual Edman degradation and hydrolysis with carboxypeptidase A.

The secondary structure of the histone fragments has been studied by circular dichroism, and we present in Table II the results that were obtained for H2A and H2B fragments. CD

Table I: Amino Acid Composition of Histone Fragments[a]

	H2A fragments			H2B fragments			H4 fragments			
amino acid	1-56[b]	1-89[c]	73-129[c]	1-83[d]	1-59[e]	63-125[e]	1-84[e]	85-102[e]	1-67[c]	69-84[c]
aspartic acid	1.1 (1)	5.6 (5)	4.9 (5)	5.2 (5)	2.1 (2)	4.0 (4)	4.3 (4)	1.0 (1)	3.3 (3)	0.3 (0)
threonine	1.0 (1)	3.1 (3)	2.9 (3)	3.0 (2)	2.1 (2)	5.6 (7)	5.8 (6)	1.0 (1)	2.1 (2)	3.6 (4)
serine	3.2 (3)	3.3 (3)	1.1 (1)	8.0 (9)	6.7 (7)	6.5 (6)	2.2 (2)	0.0 (0)	1.9 (2)	0.0 (0)
glutamic acid	4.0 (4)	7.4 (7)	6.0 (6)	6.8 (6)	4.3 (4)	6.2 (6)	6.0 (5)	1.1 (1)	4.3 (4)	1.1 (1)
proline	2.1 (2)	3.1 (3)	2.9 (3)	4.5 (5)	5.1 (5)	1.0 (1)	1.0 (1)	0.0 (0)	1.1 (1)	0.0 (0)
glycine	8.8 (9)	10.0 (10)	3.8 (4)	5.3 (5)	3.4 (3)	3.5 (3)	12.7 (13)	3.9 (4)	12.7 (13)	0.0 (0)
alanine	8.6 (9)	13.5 (14)	4.0 (4)	9.4 (9)	6.3 (6)	7.1 (7)	6.3 (6)	1.1 (1)	3.6 (3)	3.1 (3)
valine	4.9 (5)	5.3 (5)	3.0 (3)	5.4 (6)	4.6 (5)	4.1 (4)	5.9 (7)[i]	1.0 (2)[f]	4.6 (5)[i]	2.0 (2)
methionine	0.0 (0)	0.0 (0)	0.0 (0)	1.8 (2)	(f) (0)	0.0 (0)	g (0)	0.0 (0)	0.0 (0)	0.9 (1)
isoleucine	0.0 (0)	3.3 (4)[h]	4.0 (5)[h]	4.1 (4)	1.1 (1)	3.7 (4)	5.3 (6)[i]	0.0 (0)	5.5 (6)[i]	0.0 (0)
leucine	5.0 (5)	10.1 (10)	7.8 (8)	3.4 (2)	1.0 (1)	5.0 (5)	6.2 (6)	2.0 (2)	6.2 (6)	0.0 (0)
tyrosine	2.0 (2)	3.1 (3)	0.0 (0)	3.3 (4)	2.6 (3)	1.9 (2)	2.0 (2)	1.8 (2)	1.0 (1)	1.0 (1)
phenylalanine	1.2 (1)	1.3 (1)	0.0 (0)	1.9 (2)	0.0 (0)	1.8 (2)	1.0 (1)	0.9 (1)	1.0 (1)	0.0 (0)
histidine	1.0 (1)	1.9 (2)	2.7 (3)	1.9 (2)	0.9 (1)	2.0 (2)	2.1 (2)	0.0 (0)	1.1 (1)	1.0 (0)
lysine	5.1 (5)	7.0 (7)	8.9 (9)	13.9 (15)	15.8 (15)	5.7 (5)	9.9 (10)	1.2 (1)	7.8 (8)	2.1 (2)
arginine	7.8 (8)	11.6 (12)	3.1 (3)	5.1 (5)	2.9 (3)	4.8 (5)	12.1 (12)	2.1 (2)	10.9 (11)	1.2 (1)

[a] Results are expressed as numbers of amino acid residues per mole of peptide. No corrections were made for hydrolytic losses. The figures in parentheses are the numbers of residues given by the sequences of calf histones H2A (Yeoman et al., 1972), H2B (Iwai et al., 1972), and H4 (Delange, 1969). [b] Fragment generated by V8 staphylococcal protease hydrolysis. [c] Fragments obtained by cleavage at aspartyl residues. [d] Fragments generated by limited chymotryptic digestion. [e] Fragments obtained by cyanogen bromide cleavage. [f] Low value because of incomplete cleavage of a Val-Val bond during a 24-h hydrolysis. [g] Homoserine was found to be present but was not determined. [h] Low value because of incomplete cleavage of a Ile-Ile bond during a 24-h hydrolysis. [i] Low value because of incomplete cleavage of a Val-Ile bond during a 24-h hydrolysis.

Table II: α-Helix Content of Calf Thymus Histones and Histone Fragments from CD Spectra[a]

	0.01 M sodium phosphate, pH 6.5			0.01 M sodium phosphate-2 M NaCl, pH 6.5		
histone fragments	θ_{222} (deg)	% α	no. of residues in α helix	θ_{222} (deg)	% α	no. of residues in α helix
H2B (1-59)	−800	2.7	1.6	−1900	6.3	3.7
H2B (1-83)	−4700	15.7	13	−8800	29.3	24.4
H2B (63-125)	−4300	14.3	9.0	−11100	37.0	23.3
H2B (1-125)	−5150	17.2	21.5	−9000	30.0	37.5
H2A (1-56)	−900	3.0	1.7	−4700	15.7	8.8
H2A (1-89)	−5100	17.0	15.1	−6400[b]	21.3[b]	19.0[b]
H2A (73-129)	−3500	11.7	6.7	−5000	16.7	9.5
H2A (1-129)	−6000	20.0	25.8	−8600	28.7	37.0

[a] A 100% α-helical ellipticity value of −30 000° at 222 nm was taken from Chen et al. (1974). [b] Values obtained in 0.01 M sodium phosphate-1.5 M NaCl, pH 6.5.

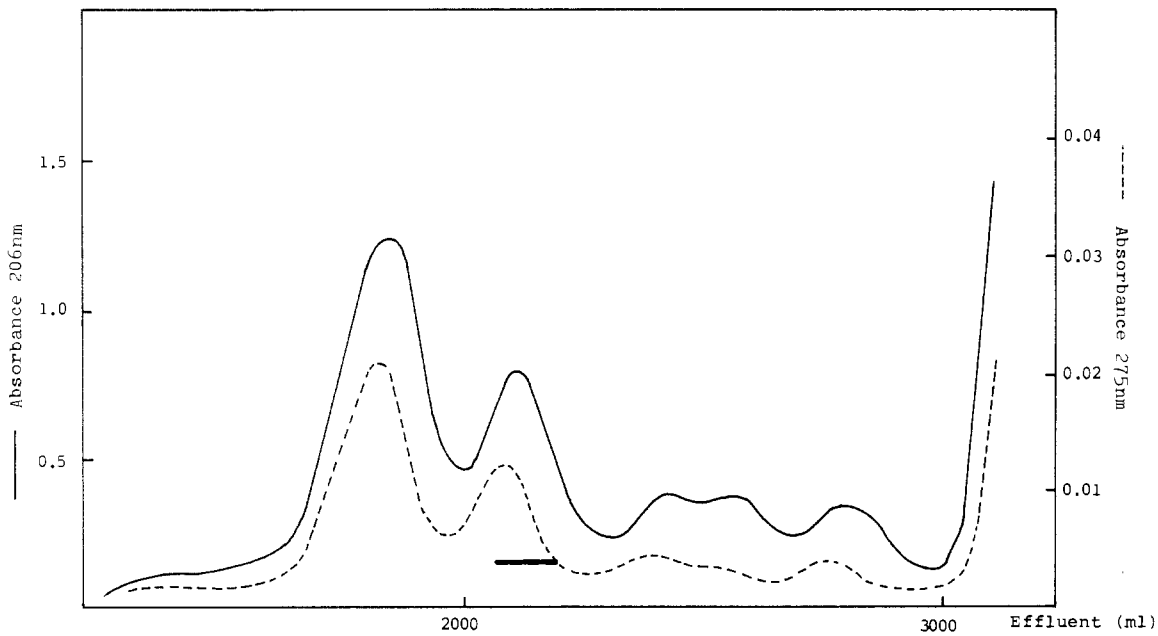

FIGURE 1: Sephadex G-100 chromatography of limited chymotryptic digest of calf histone H2B. The two columns (90 × 5 cm) coupled in series were equilibrated and eluted with 10 mM HCl. The eluate was monitored continuously at both 206 and 275 nm with two LKB Uvicord S (Bromma Sweden) columns coupled in series. The path length of the cell was 2.5 mm. The flow rate was 41 mL/h, and 13.7-mL fractions were collected. The fraction containing fragment 1–83 was pooled according to solid bar.

Table III: Characteristic Absorptions of A- and B-Type Geometries of DNA[a]

A-type helix		B-type helix	
pol	cm^{-1}	cm^{-1}	pol
⊥	1708	1715	⊥
		1280	⊥
⊥	1240	1220	0
0	1185		
//	1090	1085	⊥
⊥	1055	1050	⊥
//	882		
0	860	835	//
⊥	805		

[a] Pol, polarization in case of oriented samples; ⊥, perpendicular; //, parallel; 0, nondichroic.

spectra at different ionic strengths will be presented elsewhere. Data previously published concerning H4 fragments (Crane-Robinson et al., 1977) were used for the discussion of our IR results.

Analysis of the Vibrational Spectroscopy Data. The A and B family geometries of the DNA in the complexes can be easily characterized by infrared spectroscopy by the positions, relative intensities, and polarizations of many absorption bands. Table III summarizes the main differences of the spectra of these two conformations. During the B → A conformation transition, the 1715-cm^{-1} band, characteristic of the DNA base pairing, is shifted to 1708 cm^{-1}. The nondichroic 1220-cm^{-1} absorption due mainly to the antisymmetric OPO^- stretching vibration becomes perpendicularly polarized and is shifted to 1240 cm^{-1}. The 1280-cm^{-1} band is no longer observed whereas a new absorption at 1185 cm^{-1} is present. The strong absorption involving the symmetric stretching of the phosphate groups at 1085 cm^{-1} is shifted to 1090 cm^{-1} and becomes parallelly polarized instead of perpendicularly. Finally, in the phosphodiester chain vibrations below 1000 cm^{-1}, the 835 cm^{-1} band is no longer detected, while three absorptions at 882 (strongly parallel), 860 (nondichroic), and 805 cm^{-1} (strongly perpendicular) are observed.

It is thus possible to identify the geometry of the DNA helix in the nucleoproteic complexes. Moreover, the relative amount of both geometries in the case of the coexistence of the two conformations can be computed by using either the ratio of the integrated absorptions at 835 and 860 cm^{-1} or the determination of a very sensitive conformational probe, the orientation of the dipole transition moment of the absorption band at 1090 cm^{-1} with respect to the double helix axis θ_{1090}. Both methods issue similar results. A simple computation allows us then to obtain the number of DNA base pairs stabilized at low relative humidity in a B geometry by each histone fragment [for details, see Liquier et al. (1977)]. This has been systematically obtained for complexes prepared with H2A, H2B, and H4 fragments and exposed to relative humidities (RH) varying between 98 and 47%. The spectra of all the complexes obtained in high relative humidity conditions (RH >81%) show the same characteristic features concerning the DNA absorptions bands: in these nucleoproteins, the DNA adopts at high RH a B family form geometry. An example of such spectra is shown Figure 2 (top) in the case of a complex prepared with the (1–59) fragment of H2B (weight input ratio 1:0.24). In low relative humidity conditions (RH <71%), the spectra of the complexes differ depending on the histone fragment present in the sample. Thus, nucleoproteins reconstituted with N terminal fragments of (1–53) H4, (1–59) H2B, and (1–56) H2A present in these conditions spectra reflecting mainly an A-type geometry of the DNA (Figure 2, bottom).

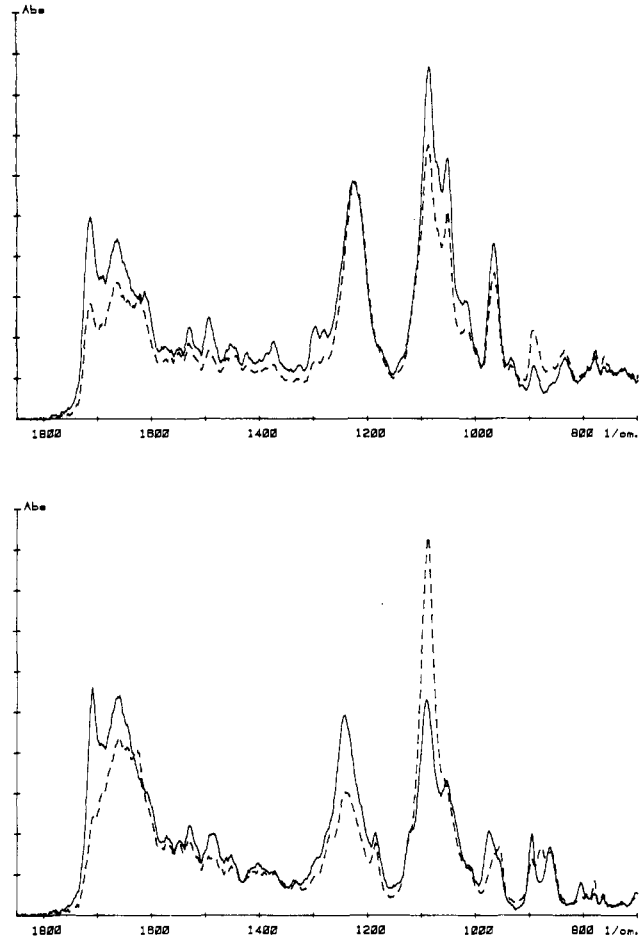

FIGURE 2: Infrared absorption polarized spectra of a DNA–H2B (1–59) complex (N-terminal fragment). Weight input ratio was 1:0.24. (Top) High relative humidity; (bottom) low relative humidity; (full line) electric vector of polarized light perpendicular to the orientation axis; (dotted line) electric vector of polarized light parallel to the orientation axis.

On the contrary, if we consider complexes obtained with histone fragments involving the central part of the protein, 1–67 (H4), 1–84 (H4), 69–84 (H4), 1–83 (H2B), 73–129 (H2A), and 1–89 (H2A), the spectra show the coexistence of the two A and B geometries of the DNA in low RH conditions, the relative amounts of these conformations depending on the DNA to protein input ratio. In Figure 3 are shown the spectra obtained at low relative humidity (RH 58%) in the 750–900-cm^{-1} region of complexes prepared with various H4 fragments in similar conditions. One can clearly observe that the two A and B geometries are present in the DNA–H4 (1–84) and DNA–H4 (69–84) complexes while almost only the A conformation is detected for the DNA–H4 (85–102) and DNA–H4 (1–53) complexes. The results obtained for the various complexes studied are presented in Table IV. We clearly observe that the different histone fragments have extremely different efficiencies as far as the stabilization of the DNA in a B geometry is concerned.

The infrared spectra of the complexes give us also informations about the protein structure of the histones. The positions and intensities of the amide I (primarily C=O stretch) and amide II (C–N stretch and N–H bend) absorptions allow to interpret the secondary structure of proteins (Chirgadze & Nevskaya, 1970a,b; Nevskaya & Chirgadze, 1976); however, the amide I band at about 1650 cm^{-1} is obscured in aqueous solutions and hydrated films by the 1640-cm^{-1} band of liquid water. Besides, in nucleoproteic complexes strong absorptions of DNA are observed in the same spectral

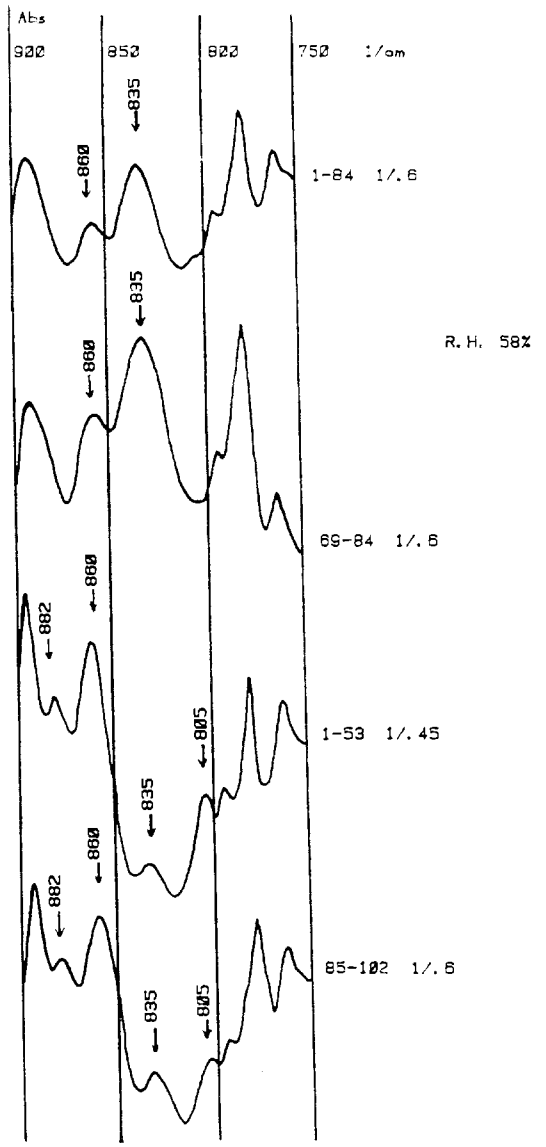

FIGURE 3: Absorption spectra in the phosphodiester chain vibration region of DNA–H4 fragment complexes obtained in low relative humidity conditions. Arrows point out characteristic absorptions of A (882, 860, and 805 cm^{-1}) and B (835 cm^{-1}) geometries.

region. Various efforts to get around this problem have been employed, including the use of D_2O. D_2O solutions pose the additional problem of the hydrogen–deuterium exchange resulting in a change in the intensity of the undeuterated amide II band at the same frequency as the HDO band. We obtain here the spectra of the histone fragments in a hydrated form and in the presence of the DNA in the complex thanks to the computer associated to the spectrophotometer. The nucleoprotein spectra are corrected for the water contribution. The relative amounts of the different DNA geometries present in the sample are then determined by the measurement of the integrated characteristic absorptions located at 835 and 860 cm^{-1} (respectively for a B- and an A-type helix). The spectrum of the DNA present in the nucleoprotein is then computed by using "perfect A and B" memorized data. The DNA spectrum is normalized and subtracted from the spectrum of the complex, leaving the absorptions of the protein. A flat plot obtained between 1300 and 1000 cm^{-1}, where the phosphate groups of the DNA absorb strongly, is a test for the validity of the computation. An example is presented Figure 4 in the case of the DNA–H2A (1–89) complex with a DNA to protein weight input ratio of 1:0.45. We have thus observed the amide

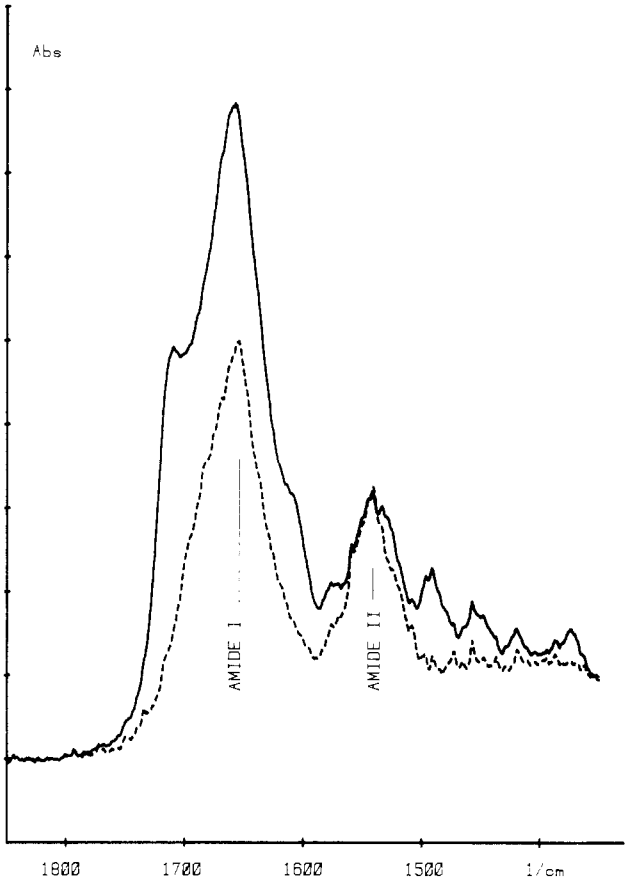

FIGURE 4: Infrared absorption spectrum of a DNA–H2A (1–89) complex. Weight input ratio was 1:0.45. Low relative humidity (66% RH) was used. (Full line) complex; (dotted line) histone in the complex.

I absorption characteristic of an α-helical conformation at 1665 cm^{-1}. Except in the case of extremely high protein to DNA ratio, no absorptions characteristic of β-sheet structure (1680 and 1625–1630 cm^{-1}) were observed.

Discussion

The simultaneous study of the protein structure and of the DNA conformational flexibility in the nucleoproteic complexes gives us an opportunity to determine precisely the important role played by the histone conformation in maintaining the DNA geometry and may serve as a model for structurally important DNA–histone interactions in the nucleosomal core particle. In fact, we shall see that the α-helical parts of the histones interact with the DNA.

It is well established that in native and reconstituted core particles, histones contain a high amount of α-helical structure (about 50%) (Cotter & Lilley, 1977). In the case of DNA–H2A, DNA–H2B, and DNA–H4 complexes, virtually no β-sheet structure can be detected by IR spectroscopy except in the case of DNA to histone ratios higher than 1:0.45. In the latter case, we must however notice that the amount of β-sheet structure is relatively small when compared to the data corresponding to the free histones and their peptides [for example, we find about 10% of β structure in the DNA–H4 complex whereas 27–30% of β structure was measured for free H4 in D_2O (Shestopalov & Chirgadze, 1976)]. This decrease in the amount of β-sheet structure is still more important in the histone belonging to native or reconstituted core particles; in these nucleoproteins almost no β structure was detected in our laboratory, in good agreement with the conclusion of previous studies of chromatin subunits (Cotter & Lilley, 1977). The α-helical and partly random conformation of the histones

Table IV: Stabilization of the DNA B Geometry by Histone Fragments Determined by Infrared Linear Dichroism[a]

complex	protein to DNA wt ratio (%)	θ_{1090} at low RH (deg)	no. of base pairs of DNA blocked in B geometry per fragment	complex	protein to DNA wt ratio (%)	θ_{1090} at low RH (deg)	no. of base pairs of DNA blocked in B geometry per fragment
DNA		49		DNA-H2B	12	48	
DNA-H4	25	50		(1–59)	24	49	6
(1–53)	45	49	0		38	51	
	55	51			50	54	
	60	49		DNA-H2B	19	54	
DNA-H4	20	52		(1–83)	33	60	24
(1–67)	40	54.5	10		52	63	
	60	56.5			67	*b*	
DNA-H4	15	53		DNA-H2B	12	49	
(1–84)	25	57	18	(1–125)	26	50	24
	30	56			40	56.5	
	60	62		DNA-H2A	15	49	
DNA-H4	10	51.5		(1–56)	30	54	
(1–102)	25	55	22		45	53	5
	35	57			60	53.5	
	45	58		DNA-H2A	14	52	
DNA-H4	10	53		(1–89)	31	57	20
(69–84)	20	55.5	5		44	58.5	
	30	59		DNA-H2A	15	51	
	40	62		(73–129)	30	58.5	14
DNA-H4	20	51			45	61	
(85–102)	40	52	<2		60	62	
	60	54.5		DNA-H2A	14	52	
				(1–129)	30	56	21
					40	57	
					50	57	

[a] For the computation method, see Liquier et al. (1977). [b] Nondichroic.

in these complexes must therefore have an important structural role in the DNA–histone interactions.

The location of α helixes has been theoretically predicted (Lewis & Bradbury, 1974) and experimentally determined by NMR, CD and IR measurements. In the case of H2A, two α-helical segments have been proposed extending from residues 47–66 and 78–88 (Bradbury et al., 1975). For H2B, our CD results in good agreement with previous estimations (Bradbury & al., 1972; d'Anna & Isenberg, 1972) allow one to localize the α-helical structure in the 59–85 region. For H4, the proposed α helixes are located between residues 55 and 67 on one hand, and 12 residues forming an α helix are expected in the 70–90 region on the other (Crane-Robinson et al., 1977).

If we consider the results concerning the complexes prepared with the N-terminal fragments of (1–56) H2A, (1–59) H2B, and (1–53) H4, which are almost completely in a random conformation, no stabilization of the DNA in a B geometry is found. The DNA in these nucleoproteins is able to undergo a total B → A transition. On the contrary, if an α-helical part of the histone is involved in the histone–DNA interaction, as, for example, in the case of the (1–67) H4 or (73–129) H2A fragment, the DNA is partially blocked in a B geometry. Histone fragments that contain most of the α-helical parts of the corresponding histone have a similar stabilizing effect on the DNA as the whole histone (20 base pairs of DNA blocked by the 1–89 fragment of H2A instead of 21 for the whole H2A molecule, 24 base pairs for the 1–83 fragment of H2B as for H2B, and 18 base pairs for the 1–84 fragment of H4 instead of 22 for H4). Moreover, the C-terminal fragment 85–102 of H4, which does not possess any α-helical structure, remains quite effectless concerning the stabilization of the DNA B form.

Our results show that the structurally important interactions do not imply the N-terminal basic regions of the histones. This is supported by trypsin- and nuclease-digestion experiments showing that the N terminal parts of the histones do not belong to the kernel of the core particle (Weintraub & Van Lente, 1974) and by the model proposed by Pardon et al. (1977) in which the N-terminal parts of the histones protrude as arms outside of the core particle. More recently, X ray diffraction diagrams of H1-depleted chromatin were interpreted as reflecting the presence of the N-terminal parts of the histones on the spacer DNA (Azorin et al., 1980), and neutron-scattering experiments on nucleosome core particles showed that about 25% of the histones are located outside of the core (Braddock et al., 1981).

The fundamental role of the α-helical structure of the protein is in good agreement with our earlier results concerning native and reconstituted core particles in which no β structure was detected and which showed the suppression of the B → A transition of the DNA in the presence of the four histones (Liquier et al., 1979). In native core particles, Shick et al. (1980) have shown that histones are bound to discrete DNA stretches of about six nucleotides long. Thus, H2A has been found to interact at positions 75, 125, and 135 (18 nucleotides), H2B at positions 25, 35, 105, 125, and (partially) 95 (24–30 nucleotides), and H4 at positions 45, 55, and 65 (18 nucleotides). Our infrared results show that the α-helical parts of these histones are responsible for the stabilization in a B form of respectively about 21, 24, and 22 DNA base pairs. It seems thus reasonable to propose that the DNA that has been found as bound to the histones is the DNA interacting with the α-helical parts of the proteins. If we consider the α-helical parts of these histones, we can observe that all the basic residues, lysine and arginine, are clustered on one side of the helix. Thus, we can imagine that an interaction between these residues and the phosphates brings the histone α helix in close contact with the DNA, stabilizes it in a B geometry, and leaves the other side of the protein α helix free for interhistone interactions.

The important role of α-helical protein structures in protein–DNA interactions has recently been proposed in specific

systems. Thus, the DNA binding subunit of CRO protein possesses two α helixes with a 2-fold symmetry, which fit precisely into the large groove of a B-DNA structure. Similarly, the CAP protein and the λ repressor both possess α-helical parts with extremely important sequence analogies, which both are supposed to interact with the DNA. Moreover, recent crystallographic studies of CRO–DNA and CAP–DNA cocrystals have shown that the DNA is locally bent by the interaction with these proteins (McKay & Steitz, 1981; Anderson et al., 1981).

It seems thus possible to propose that in the core particle, the histones stabilize the DNA in a B geometry by interactions between the α-helical parts of the proteins and the DNA. These interactions tend to bend the DNA, making it more easy for the DNA to adopt a superhelical conformation; the N-terminal parts of the histone are not implied in this folding mechanism and, though interacting with the DNA by electrostatic interactions, remain outside of the core particle.

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Supplementary Material Available

Five figures showing isolation of the histone fragments (5 pages). Ordering information is given on any current masthead page.

References

Anderson, W. F., Ohlendorf, D. H., Takeda, Y., & Matthews, B. W. (1981) *Nature (London) 290*, 754.

Azorin, F., Martinez, A. B., & Subirana, J. A. (1980) *Int. J. Biol. Macromol. 2*, 81–92.

Bradbury, E. M., Cary, P. D., Crane-Robinson, C., & Riches, P. L. (1972) *Eur. J. Biochem. 26*, 482.

Bradbury, E. M., Cary, P. D., Crane-Robinson, C., Rattle, M. W. E., Boublik, M., & Sautiere, P. (1975) *Biochemistry 14*, 1876.

Braddock, G. W., Baldwin, J. P., & Bradbury, E. M. (1981) *Biopolymers 20*, 327–343.

Chen, Y. H., Yang, J. T., & Chau, K. H. (1974) *Biochemistry 13*, 3350.

Chirgadze, Yu. N., & Nevskaya, N. A. (1970a) *Biopolymers 15*, 607–625.

Chirgadze, Yu. N., & Nevskaya, N. A. (1970b) *Biopolymers 15*, 626–636.

Cotter, R. I., & Lilley, D. H. (1977) *FEBS Lett. 82*, 63–68.

Couppez, M., Sautiere, P., Brahmachari, S. K., Brahms, J., Liquier, J., & Taillandier, E. (1980) *Biochemistry 19*, 3358–3363.

Crane-Robinson, C., Hayashi, H., Cary, P. D., Briand, G., Sautiere, P., Krieger, D., Vidali, G., Lewis, P. N., & Tom Kun, J. (1977) *Eur. J. Biochem. 79*, 535.

d'Anna, J. A., & Isenberg, I. (1972) *Biochemistry 11*, 4017.

Felsenfeld, G. (1978) *Nature (London) 271*, 115–122.

Gross, E., & Witkop, B. (1962) *J. Biol. Chem. 237*, 1856–1860.

Iwai, K., Hayashi, H., & Ishikawa, K. (1972) *J. Biochem. (Tokyo) 72*, 357–367.

Johns, E. W. (1964) *Biochem. J. 92*, 55–59.

Johns, E. W. (1967) *Biochem. J. 105*, 611–614.

Lewis, P. N., & Bradbury, E. M. (1974) *Biochim. Biophys. Acta 336*, 153.

Liquier, J., Taboury, J., Taillandier, E., & Brahms, J. (1977) *Biochemistry 16*, 3262–3266.

Liquier, J., Gadenne, M. C., Taillandier, E., Defer, N., Favatier, F., & Kruh, J. (1979) *Nucleic Acids Res. 6*, 1479–1493.

Lowry, O. H., Rosebrough, N. J., Farr, A. L., & Randall, R. J. (1951) *J. Biol. Chem. 193*, 265.

McKay, D. B., & Steitz, T. A. (1981) *Nature (London) 30*, 744.

Michalski-Scrive, C., Aubert, J. P., Couppez, M., Biserte, G., & Loucheux-Lefebvre, M. H. (1982) *Biochimie 64*, 347–355.

Nevskaya, N. A., & Chirgadze, Yu. N. (1976) *Biopolymers 15*, 637–648.

Panyim, S., & Chalkley, R. (1969) *Arch. Biochem. Biophys. 130*, 337–346.

Pardon, J. F., Worcester, D. L., Wooley, J. C., Cotter, R. I., Lilley, D. M. J., & Richards, B. M. (1977) *Nucleic Acids Res. 4*, 3199–3214.

Schroeder, W. A., Shelton, J. R., Shelton, J. B., Cormick, J., & Jones, R. T. (1963) *Biochemistry 2*, 992–1008.

Shestopalov, B. V., & Chirgadze, Yu. N. (1976) *Eur. J. Biochem. 67*, 123–128.

Shick, V. V., Belyavsky, A. V., Bavykin, S. G., & Mirzabekov, A. D. (1980) *J. Mol. Biol. 139*, 491–517.

Taillandier, E., Taboury, J., Liquier, J., Gadenne, M. C., Champagne, M., & Brahms, J. (1979) *Biopolymers 18*, 1877–1888.

Weintraub, H., & Van Lente, F. (1974) *Proc. Natl. Acad. Sci. U.S.A. 71*, 4249–4253.

Weisbrod, S. (1982) *Nature (London) 297*, 289–295.