Site and Role of the N-Terminal Fragment of the Nucleosomal Core Histones in Their Binding to Deoxyribonucleic Acid As Determined by Vibrational Spectroscopy†

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ABSTRACT: The site and role of the binding of the 1–53 N-terminal part of H4 on DNA have been studied by optical spectroscopy. The structure of the 1–53 H4 fragment determined by vacuum ultraviolet circular dichroism and infrared spectroscopy is essentially aperiodic. The site of the interaction between the fragment and free DNA is localized by Raman laser spectroscopy in the small groove of the DNA, similar to the interaction site of the whole histone with DNA in nucleosomes. Infrared linear dichroism measurements show that the two 1–53 and 54–102 H4 fragments play a very important role in the histone–DNA interactions, but the roles are extremely different: the N-terminal part of the histone remains effectless on the DNA conformational flexibility and it is proposed that the structurally important interaction occurs between the globular part of the histone and the DNA. The N-terminal fragment appears to be responsible for finding the correct place on the DNA of the nucleosomal core particles.

Vibrational spectroscopy is a powerful tool for the investigation of the secondary structure of nucleic acids and proteins and the localization of the interactions between the different constituents of chromatin. This method is successful to study the influence of different regions of histones on chromatin DNA. Histones can be cleaved into fragments containing various numbers of peptides. The structure of such fragments has been studied by NMR (Crane-Robinson et al., 1977), and complexes formed with DNA have been examined by circular dichroism and thermal denaturation (Adler et al., 1975).

It has recently been proved by infrared and Raman spectroscopy (Liquier et al., 1979; Goodwin & Brahms, 1978) that DNA in chromatin is in a high humidity B-type conformation. Infrared studies of DNA interactions with the four histones belonging to the core particle, single or preassociated, have shown that in such complexes the DNA also remains blocked in a B-type conformation, in contrast to what is observed for free DNA which can easily adopt two different types of geometries (A and B) (Pilet & Brahms, 1973; Liquier et al., 1977; Taillandier et al., 1979). The localization of the interaction responsible for this loss of conformational flexibility is unknown. In the different models proposed for the chromatin structure it is usually accepted that the histone–DNA interactions occur between the N-terminal parts of the histones and the DNA, whereas the more hydrophobic parts of the histones interact between themselves to constitute a nucleosomal protein core. Thus, it seems important to examine the role and influence of this N-terminal fragment of histones on the DNA secondary structure.

In this study we have used the N-terminal fragment of H4 (peptides 1–53). The question arises as to whether the interaction between DNA and a histone fragment is the same as that between DNA and the intact histones of the proteic core in chromatin. It is found by Raman spectroscopy that the site of fixation is similar in the interaction between DNA and the histone fragment to that in chromatin. Infrared linear dichroism allows us to examine in detail the possible secondary structures of DNA when associated with this H4 fragment. We show that when the 1–53 fragment of H4 is bound to the DNA, the latter remains able to undergo conformational transitions. Moreover, we have examined the secondary conformation of the protein by ultraviolet circular dichroism and infrared spectroscopy and confirmed that the 1–53 fragment of H4 is essentially in an aperiodic random structure.

Materials and Methods

Isolation of Histone H4. Calf histone H4 was obtained directly in pure form through a slight modification of the procedure of Johns (1967). The extract guanidine hydrochloride–ethanol at pH 7.0 was treated by 1.5 volumes of acetone (instead of 1 volume as recommended in the original method) in order to ensure a complete precipitation of the histone H2A in the fraction F2a2. The fraction F2a1 was recovered by adding 1.5 volumes of acetone to the supernatant. This fraction F2a1 was indeed constituted of pure histone H4.

Hydrolysis of Histone H4 with Staphylococcal Protease. Histone H4 (50 mg) was dissolved in 10 mL of 0.05 M ammonium acetate buffer, pH 4.0, and hydrolyzed with staphylococcal protease (Miles) at 37 °C for 18 h, using an enzyme/substrate ratio of 1:50. The hydrolysate was then frozen, lyophilized, and fractioned on a Sephadex G-100 column (200 × 2.5 cm) equilibrated and eluted with 0.01 M HCl. The fractions thus obtained were analyzed by polyacrylamide gel electrophoresis at pH 2.7 in 2.5 M urea (Panyim & Chalkley, 1969) by using 17% acrylamide.

Complex Preparation. H4 fragment was dissolved in 10^{-3} M HCl. An appropriate amount of NaCl solution was added so as to obtain a final 2 M NaCl–histone solution immediately before adding the DNA solution. DNA (salmon sperm) was purchased from Serva and dissolved in 2 M NaCl. DNA concentrations (12.5 mg/mL) were determined by ultraviolet spectroscopy, assuming $OD_{260nm}^{1cm} = 20$ for a 1 mg/mL solution. The mixtures were then dialyzed in the cold room. Dialysis steps were 1.5 M NaCl, 0.75 M NaCl, 0.3 M NaCl, and a final adequate NaCl solution (between 3 and 5×10^{-3} M NaCl) which ensures an NaCl/DNA weight ratio of 6%

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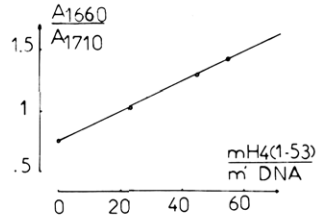

FIGURE 1: Determination of the composition of the H4 (1–53)–DNA complexes.

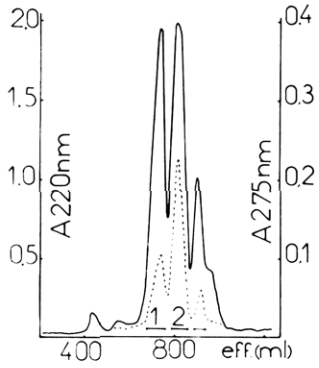

FIGURE 2: Sephadex G-100 chromatography of the staphylococcal protease hydrolysate of calf histone H4. The column (200 × 2.5 cm) was equilibrated and eluted with 0.01 N HCl. The flow rate was 14.4 mL/h, and 7.2-mL fractions were collected. Fractions were pooled according to the solid bars. Peak 1 corresponds to peptide SP-1.

in the sample. Under these salt conditions it has previously been shown that pure DNA is able to undergo a complete reversible B → A conformational transition. Protein/DNA ratios were estimated by infrared spectroscopy using the amide I and amide II bands for the protein and a characteristic DNA absorption located at 1710 cm^{-1} (Figure 1). The weight input ratios of the prepared complexes were 0.25:1, 0.35:1, 0.45:1, 0.55:1, and 0.65:1. For Raman laser scattering measurements, the lowering of the salt concentration of the initial mixture of N-terminal fragment with DNA in 2 M NaCl was obtained by dilution with a buffer containing 20 mM Tris, pH 8, and 0.2 mM EDTA, followed by a dialysis against 5 mM NaCl, 20 mM Tris, pH 8.0, and 0.2 mM EDTA. Protein/DNA ratios (w/w) of the prepared complexes were 0.20–0.50:1, i.e., 4–10 molecules of the fragment per 200 base pairs of DNA.

Spectroscopic Measurements. The infrared spectra were recorded between 4000 and 700 cm^{-1} with a Perkin-Elmer 180 double-beam ratio recorder grating spectrophotometer equipped with a grid polarizer. The polarizer was placed in the common beam and rotated at ±45° with respect to the slits and the gratings. The samples were deposited on Irtran 2 windows and oriented by undirectional stroking. The obtained films were sealed in a constant relative humidity cell and oriented at 45° with respects to the slits. The relative humidity of the cells was monitored by saturated salt solutions and controlled by the water absorption measurement at 3400 cm^{-1}.

Raman laser scattering measurements were performed as described previously (Goodwin & Brahms, 1978). Vaccum ultraviolet circular dichroism was measured by applying a previously described approach (Brahms et al., 1977; Brahms & Brahms, 1980). The vacuum ultraviolet circular dichroism measurements were performed on the N-terminal fragment dissolved in D_2O.

Results

Characterization of the Histone Fragment. The Sephadex G-100 elution pattern of the histone H4 hydrolyzed with staphylococcal protease (see Materials and Methods) is shown in Figure 2. Three major fractions were obtained. Fraction 1, which appears as a broad band on gel electrophoresis (Figure 3, slot 3), corresponds to the amino-terminal half of the histone H4 (peptide SP-1, residues 1–53), assessed by the amino acid composition (Table I). This H4 fragment, produced by the specific cleavage of the bond between Glu-53 and Thr-54, is 50% acetylated at Lys-16 (De Lange et al., 1969). It contains 17 of the 27 basic residues present in histone H4, which gives it a markedly basic character. The peptide 1–53 is also characterized by two basic sequences

$$\underset{16}{\text{Lys(Ac)}}\text{-Arg-His-Arg-}\underset{20}{\text{Lys(Me)}} \qquad \underset{35}{\text{Arg}}\text{-Arg-Leu-Ala-Arg-}\underset{40}{\text{Arg}}$$

which makes it of pecular interest with a view of studying histone–DNA interactions.

Vacuum Ultraviolet Circular Dichroism Studies. The results of vacuum ultraviolet circular dichroism allow us to determine the secondary structure of the 1–53 fragment in

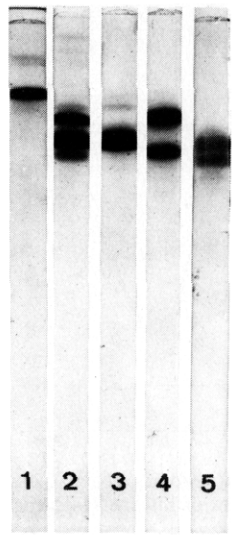

FIGURE 3: Polyacrylamide gel electrophoresis at pH 2.7 in 2.5 M urea of staphylococcal protease peptides from calf histone H4. Electrophoresis was performed at 1.5 mA/tube for 90 min. Gels were stained for 30 min with 1% amido black 10B in acetic acid–ethanol–water (1:2:7 v/v/v). Slot 1, histone H4; slot 2, staphylococcal protease hydrolysate of histone H4; slot 3, peak 1; slot 4, peak 2; slot 5, peak 3.

Table I: Amino Acid Composition of Peptide SP-1 Obtained by Staphylococcal Protease Cleavage of Calf Histone H4[a]

amino acids	H4-SP-1 1–53	histone H4
Asp	2.4 (2)	5
Thr	1.1 (1)	7
Ser	1.9 (2)	2
Glu	2.8 (3)	6
Pro	1.2 (1)	1
Gly	11.9 (12)	17
Ala	3.3 (3)	7
Val	2.2 (2)	9
Met	0.0 (0)	1
Ile	4.7 (5)	6
Leu	4.3 (4)	8
Tyr	1.2 (1)	4
Phe	0.0 (0)	2
His	1.2 (1)	2
Lys[b]	7.1 (7)	11
Arg	9.1 (9)	14
no. of residues	(53)	102

[a] Results are expressed as the number of amino acid residues. Figures in parentheses are the nearest integers. [b] Including N^{ϵ}-methyllysine.

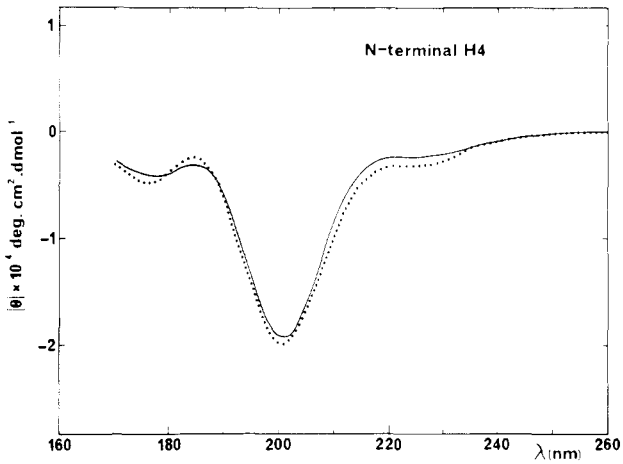

FIGURE 4: Vacuum ultraviolet circular dichroism spectrum of N-terminal 1–53 fragment of histone H4 measured in D_2O solution, neutral pH.

D_2O, which appears essentially in an aperiodic, random conformation (Figure 4). The quantitative analysis of the conformational parameter indicates that the N-terminal fragment is characterized by the following contributions: 65% of aperiodic random structure; 15% of β turns; 14% of α-helical and 6% of β structures. This seems to be in agreement with its amino acid composition (Table I), indicating that glycines and proline, α-helix breakers, are in a very important amount (25 mol %) and that positively charged arginines and lysines are concentrated in the N-terminal portion of the molecule, bringing a high positive charge density. This predominant contribution of aperiodic structure is essentially in agreement with the previous qualitative investigation performed in a limited ultraviolet spectral region by Lewis et al. (1975) on a smaller fragment (1–23). For a much larger N-terminal fragment (1–84) Adler et al. (1975) found 60% of the residues in random conformation and 40% in β structure, which according to the authors reflects an overestimate of the β structure by the method used. The circular dichroism spectra show that the conformation of the 1–53 N-terminal fragment was not changed upon increasing the ionic strength to 0.1 M KF and by formation of complexes with DNA.

Raman Laser Scattering Studies. Raman laser scattering investigations were performed on complexes of N-terminal fragment–DNA in solution and in fibers. The Raman laser spectra of N-terminal fragment–DNA complex at low protein/DNA ratios (r = 0.2) in H_2O solution and D_2O solution are shown in parts a and b of Figure 5. The formation of complexes is reflected in some changes of the intensity of Raman bands characteristic of base vibrations. Quantitative comparison of the intensity of these bands with respect to that of the phosphate O···P···O symmetric stretching vibration at $\sim$1094 cm^{-1} leads to the following observations.

(1) The intensity of the band at $\sim$1490 cm^{-1} is not changed and remains essentially identical with that of free DNA. This band is assigned predominantly to the guanine ring vibrations (Petitcolas, 1971) involving N7–C8 stretching vibrations with a relatively small contribution from adenine (Goodwin & Brahms, 1978). This indicates that the N7 position of guanine and the large groove of DNA are not the sites of interaction with the N-terminal fragment of H4.

(2) A reduction of the intensity of the 1578-cm^{-1} band has been observed in Raman spectra of complexes of N-terminal fragment when compared with spectra of DNA. The 1578-cm^{-1} Raman band results from purine base vibrations and in DNA has been assigned predominantly to adenine vibrations

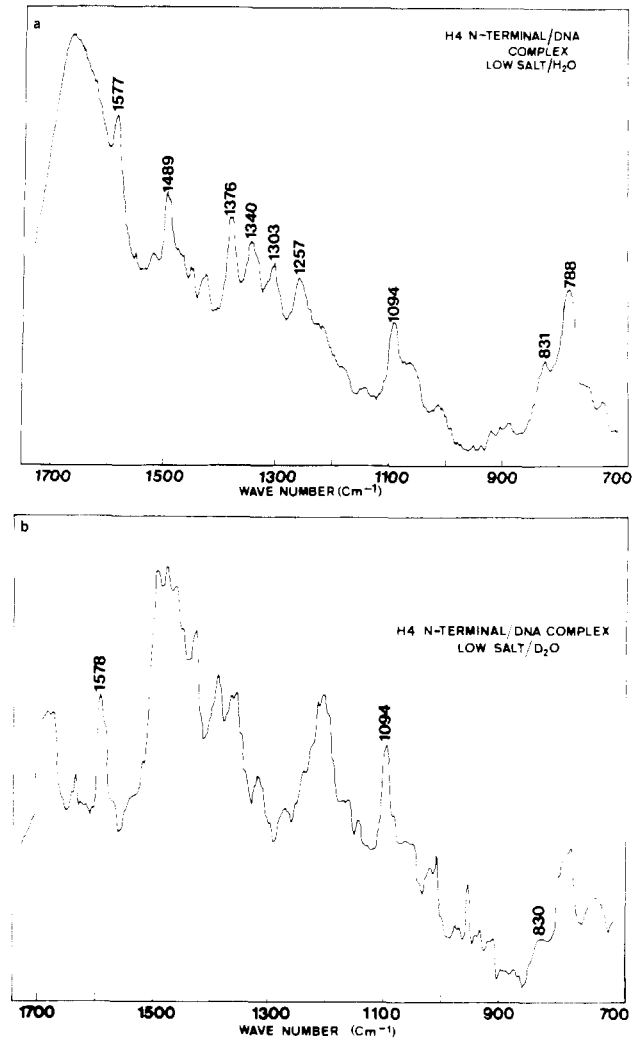

FIGURE 5: (a) Raman spectrum of the H4 N-terminal fragment–DNA complex in the ratio of 4 molecules of fragment per 200 base pairs in H_2O solution. Reconstituted complex (see text) was exposed to a final dialysis against 5 mM NaCl, 20 mM Tris, pH 8.0, and 0.2 mM EDTA. (b) Raman spectrum of H4 N-terminal fragment–DNA complex in D_2O solution. The protein/DNA ratio is as in spectrum a, and the final dialysis was achieved against D_2O solution.

Table II: Relative Intensities of the Raman Bands at 1578 and 1490 cm^{-1} with Respect to the Phosphate 1094-cm^{-1} Band

sample	1578 cm^{-1}	1490 cm^{-1}
DNA	1.35	1.15
N-terminal fragment–DNA complex (r = 0.2)	1.1	1.1
N-terminal fragment–DNA complex (r = 0.5)	1.0	0.9

(Petitcolas, 1971) involving the N3 position of adenine. A similar decrease in intensity of the 1578-cm^{-1} band of DNA was observed in native and reconstituted nucleosomes (Goodwin et al., 1979; S. K. Brahmachari and J. Brahms, unpublished results). This strongly suggests that the binding of the N-terminal fragment of H4 to DNA is occurring similarly in our complexes and in nucleosomes.

The complex, N-terminal fragment 1–53 of H4 histone with DNA, was also prepared at a higher protein/DNA ratio, r = 0.5, i.e., 10 molecules of fragment 1–53 of H4 per 200 base pairs of DNA. This concentration corresponds to an excess of protein if compared to the expected protein/DNA ratio in nucleosomes. Under this condition the formation of the

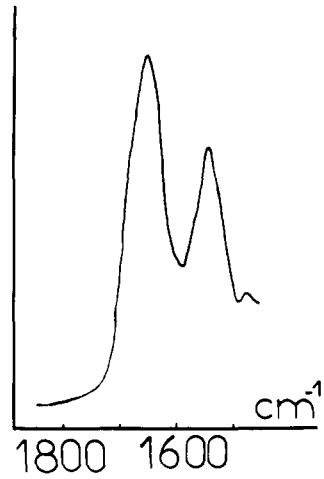

FIGURE 6: Infrared absorption spectrum in the amide I and amide II regions of the H_4 1-53 fragment bound to the DNA.

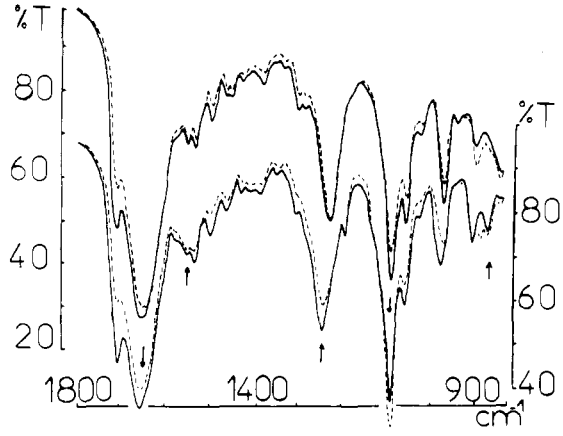

FIGURE 7: Infrared dichroic spectra of a H4 (1-53)-DNA complex (input weight ratio 0.45:1). Top: high relative humidity spectrum. Bottom: low relative humidity spectrum. (—) Electric field polarized perpendicularly to the orientation axis; (- - -) electric field polarized parallelly to the orientation axis. Arrows indicate characteristic absorptions (from left to right): amide I, amide II, phosphate antisymmetrical and symmetrical valence vibrations, and phosphodiester chain skeleton vibration.

complex leads to a slightly more pronounced decrease of the intensity of the 1578-cm^{-1} band; moreover, the 1490-cm^{-1} band has its intensity significatively reduced, with respect to the phosphate band (Table II). This strongly suggests that under the condition of an excess of N-terminal fragment molecules, not only the sites of attachement in the small groove involving N3 of adenine are occupied but also the sitelike N7 position of guanine (i.e., the 1490-cm^{-1} band) in the large groove is the site of interaction.

The Raman laser spectra of all complexes in solution and in fibers at high relative humidity show a band at ~830 cm^{-1} characteristic of the phosphodiester stretching vibration of DNA (Erfurth et al., 1975; Goodwin & Brahms, 1978) which is essentially similar to that of free DNA under the same conditions of relative humidity.

Infrared Linear Dichroism Studies. (1) Protein Conformations. In the studied spectral region two main absorptions are observed: the amide I and amide II bands. Figure 6 shows the spectrum of the protein bound to the DNA obtained by differential spectroscopy or recalculated with the help of a computer. The amide I absorption at 1650 cm^{-1} presents a symmetrical profile with no significative shoulder detected either at 1625 cm^{-1} or at 1675 cm^{-1}, usually considered as due

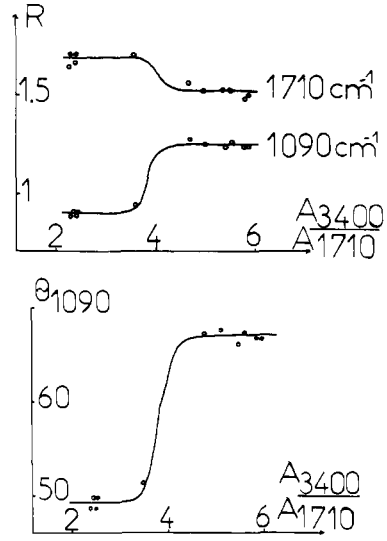

FIGURE 8: Variation of the dichroic ratios (top) and orientation parameter (bottom) of a H4 (1-53)-DNA complex (weight ratio 0.45:1) with relative humidity.

Table III: Composition and Angular Parameter of H4 (1-53)-DNA Complexes Determined by Infrared Spectroscopy

protein/DNA wt ratio	protein/DNA ratio determined by IR spectroscopy: amide I A_{1660}/A_{1710}	protein/DNA ratio determined by IR spectroscopy: amide II A_{1550}/A_{1750}	Θ_{1090} at low rel humidity
0	0.83 ± 0.03	0	49.5
25	1.02 ± 0.04	0.13 ± 0.02	50
45	1.30 ± 0.05	0.28 ± 0.06	49
55	1.42 ± 0.03	0.62 ± 0.02	51
60	1.56 ± 0.04	0.66 ± 0.03	49
65	1.67 ± 0.02	0.70 ± 0.03	ppt

to a β-sheet structure. Similarly, no shoulder is observed on the amide II band located at 1540 cm^{-1} (Chirgadze & Nevskaia, 1976). This is in good agreement with our data issued by circular dichroism; the structure of the N-terminal fragment bound to the DNA is essentially a random conformation.

(2) DNA Conformations. Figure 7 shows the dichroic spectra of a H4 (1-53)-DNA complex at high and low relative humidities. Details about the infrared linear dichroism measurements and calculations have been given previously (Pilet & Brahms, 1973; Liquier et al., 1977). Qualitatively the observation of the spectra shows that the DNA bound to the N terminal of H4 is in a B-family-type conformation at high relative humidity (top) and an A-type conformation at low relative humidity (bottom): the absorptions at 1710, 1230, and 1090 cm^{-1} are respectively polarized perpendicularly, nondichroicly, and perpendicularly in the first case and perpendicularly, perpendicularly, and parallelly in the second case; the phosphodiester deformation modes below 1000 cm^{-1} show that the B → A transition is totally observed [presence of a band at 835 cm^{-1} in the B form (top) and at 860 cm^{-1} in the A form (bottom)].

Quantitatively the measurement of the dichroic ratios of the 1090-cm^{-1} band allows us, when the relative humidity of the sample is modified, to follow the variation of the orientation of the transition dipole moment with respect to the helix axis (Figure 8). The values calculated for the different H4 (1-53)-DNA complexes are presented Table III. It is clear that the low relative humidity geometry of the DNA is not modified by the presence of the N-terminal histone fragment. The same

computation shows that the high relative humidity conformation of the DNA in the complexes belongs to the B family.

Discussion

The present study of the interactions of the N-terminal fragment of H4 with DNA may serve as a model for the understanding of the structural organization in chromatin. We have found that the isolated N-terminal fragment binds to DNA in a similar manner and in a similar region of the DNA as when it belongs to the reconstituted or native whole nucleosomal core.

The Raman spectra of reconstituted and native nucleosomes exhibit a decrease of the relative intensity of the 1578-cm^{-1} band whereas the 1490-cm^{-1} band is not changed (Goodwin et al., 1979; S. K. Brahmachari and J. Brahms, unpublished results). These two bands at 1490 and 1578 cm^{-1} are assigned in DNA to guanine and adenine, respectively (Peticolas, 1971). On the basis of Raman spectra of DNA methylated by dimethyl sulfate and the previous methylation data of Maxam & Gilbert (1977) and Lawley & Brookes (1963), the bands at 1490 and 1578 cm^{-1} were assigned predominantly to vibrations involving N7 of guanine and N3 of adenine, respectively. Thus, the site of interaction of the N-terminal fragment involves N3 of adenine exposed in the narrow groove whereas the large groove remains free.

The similitude between the binding of the N-terminal fragment of H4 and the whole H4 molecule to DNA may be explained by the very dissymmetrical structure of H4 (see Table I). It contains 17 of the 27 basic residues of H4. On the basis of previous sequence determination (De Lange et al., 1969; Ogawa et al., 1969), it was largely accepted that the N-terminal fragment of H4, which is the cationic part, binds to DNA. NMR studies of histones essentially confirmed this representation of N-terminal parts of histones as responsible for binding to DNA (Bradbury & Rattle, 1972; Lewis et al., 1975). Previous experiments indicated that after trypsin digestion there is an increased sensitivity of chromatin to the digestion by staphylococcal nuclease (Weintraub & Van Lente, 1974). The trypsin digestion of nucleosome (PS) particles by Sahasrabudhe & Van Holde (1974) indicated that DNA becomes unfolded and its hydrodynamic behavior is approximately that expected for an extended rodlike DNA covered with some remaining proteins. It appears thus that the N-terminal fragment protects DNA against staphylococcal nuclease digestion, whereas its digestion by trypsin leads to the loss of the folded DNA structure.

The present experiments suggest that this N-terminal part, which has a very flexible, essentially random, conformation as shown by circular dichroism and infrared determinations, not only binds to DNA like an arm but also has the function to choose its place on DNA following the narrow groove. This mechanism occurs independently, whether the nucleosomal particles are entire or whether only the N-terminal fragment is present. Despite the fact that the binding of nucleosomes to DNA is not sequence specific (Prunell & Kornberg, 1978; Prunell, 1979), this interaction occurs in a particular region of DNA, i.e., in its narrow groove. This may have an important biological role in view of the possible sliding or movement of nucleosomes (Steinmetz et al., 1978; Weischet, 1979), and this mechanism may ensure the finding of a correct position on DNA.

Vacuum ultraviolet circular dichroism allows a very previse analysis of the conformation of the 1–53 fragment and detects essentially an aperiodic random conformation and only a slight presence of β-turn conformation and α and β structures. The β-sheet structure is very well characterized by infrared spectroscopy. In the spectra of DNA–chromosomic nonhistone protein complexes we detect this structure in the amide I spectral region by two strong absorptions distant of $\sim$50 cm^{-1} at 1675 and 1625 cm^{-1}. These two characteristic frequencies are very clearly different from those of α helixes or random structures which are observed between 1650 and 1655 cm^{-1}.

The infrared spectra of complexes between DNA and whole H4 as well as the 1–84 fragment of H4 (for protein/DNA weight ratios above 0.25:1) show such β-structure characteristic absorptions (unpublished results). On the contrary, the infrared method sensibility does not allow us to detect any β structure in the complexes with the 1–53 fragment of H4. The β structure is therefore mostly located beyond residue 53 in H4. This is in good agreement with NMR studies which show that among the 102 residues of H4 the structured parts (α helix and β structure) are located in the 53–102 region.

Each part of the histone H4 has a different role in the histone–DNA interaction. The infrared spectroscopy shows that the effect of the binding of this H4 fragment to DNA is very different from that of the whole H4 molecule. In the latter case, the B $\rightarrow$ A conformational transition is progressively inhibited with increasing histone/DNA ratios. One H4 molecule blocks 2.5 DNA helix turns in a B-type conformation. On the contrary, the presence of only the 1–53 N-terminal part does not modify the conformational flexibility of the DNA which remains able to adopt any of the two A and B configurations. Thus, the two fragments 1–53 and 54–102 play a totally different structural role in the DNA–histone interactions. The strong electrostatic binding between the phosphates of the DNA and the charged groups of the lysine and arginine residues of the N-terminal fragment is not responsible for the loss of the DNA flexibility. This is in good agreement with our results concerning the very lysine-rich histones H1 (Taillandier et al., 1979) and H5 (unpublished results) which remain without effect on the DNA flexibility, as well as the structures of complexes between DNA and basic polypeptides (Liquier et al., 1975): polyarginine and polylysine bound to DNA leave it free to undergo all the possible conformational transitions. Therefore, it is the globular part strongly structured α helix and the partial β sheet of the histone H4 54–102 which stabilize the DNA in the high humidity (B type) conformation.

In conclusion, the binding of the N-terminal fragment of H4 is shown to occur to the similar DNA region as in the whole nucleosomes. It is shown by infrared investigation that the binding of the N-terminal fragment has no effect on the conformational flexibility of DNA in contrast to the effect of the globular nucleosomal core which stabilizes the DNA in its high humidity form. This interaction of the N-terminal fragment with DNA represents a general model in nonspecific protein–DNA interactions which can also be examplified by other nonspecific interactions occurring in biology, and this study may help in better understanding of the biological processes, such as transcription in eukaryotic cells.

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Concentration-Dependent Influence of Various Cytochalasins and Chaetoglobosins on the Phalloidin-Induced Polymerization of G-Actin in 0.6 M Potassium Iodide†

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ABSTRACT: Phalloidin, a bicyclic peptide from the poisonous *Amanita phalloides* mushroom, stimulates the viscosimetrically determined polymerization of G-actin to F-actin in 0.6 M potassium iodide, a medium in which spontaneous polymerization does not occur. The cytochalasins B, D, E, and G (CB, CD, etc.) and the chaetoglobosins A, B, C, E, F, and J (Ch-A, Ch-B, etc.) have been found to influence the rate of polymerization in different ways, depending on their chemical structure and concentrations applied. Class I cytotoxins, e.g., CB, CG, Ch-C, Ch-E, and Ch-F, which exert on F-actin a weak degradative power (DP, "Spudich effect"), increase the polymerization rate when present in a ratio of 4 mol to 1 mol of actin. Polymerization is slightly enhanced or retarded at molar ratios ranging from 0.04:1 to 0.4:1. Class III cytotoxins of strong degradative power, i.e., high affinity for actin, such as CE, Ch-B, and Ch-J, decrease the polymerization rate at a molar ratio of 4:1 during the first 30–40 min; however, they increase it at a molar ratio of 0.4:1. Members of class II (CD, Ch-A) exert an effect that can be interpreted as a combination of the effects of class I and class III cytotoxins. An explanation of this difference in behavior is offered on the basis of experiments on F-actin degradation by Ch-J and its reconstitution by phalloidin in the presence of Ch-J.

Cytochalasin B (CB),[1] a cytotoxic mold metabolite, inhibits microfilament-dependent functions of eukaryotic cells (Wessels et al., 1971; Spudich, 1973) by destabilizing F-actin, as observed by viscosimetry. The degradation does not lead to G-actin monomers; instead the rate of G-actin polymerization is increased when CB is present at a ratio of about 1 mol to 1 mol of actin (Löw & Dancker, 1976). In concentrations 5–10 times smaller, CB lowers the increase in viscosity of a solution of G-actin as compared to the control (Dancker & Löw, 1979).

Recently, Löw et al. (1979) reported on the influence of additional cytochalasins and of several chaetoglobosins (Natori, 1977) on the rate of polymerization of G-actin from rabbit muscle. They found that in a molar ratio of 0.4:1 mol of actin each of the cytotoxins investigated, CB, CD, CE, and CG, as

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[1] Abbreviations used: CB, CD, etc., cytochalasin B, D, etc.; Ch-A, Ch-B, etc., chaetoglobosin A, B, etc.; DP, degradative power (on F-actin); PHD, phalloidin.

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