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Form of DNA and the nature of interactions with proteins in chromatin

D.C. Goodwin and J. Brahms

Institut de Recherche en Biologie Moléculaire, Université Paris VII, 2 Place Jussieu Paris 5°, France

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ABSTRACT

Studies of native chromatins and of isolated nucleosomes (from calf thymus) show that the DNA is in the B form or modified B form. This was determined by Raman spectroscopy of chromatins, of nucleosomes (from calf thymus) and of DNA fibres and directly correlated with X-ray diffraction studies. The Raman spectra of three forms of DNA (A, B and C) have been characterized in fibres both by X-ray diffraction and Raman spectroscopy on the same sample. In particular, the Raman spectrum of the C form of DNA is characterized by a band of about 870 cm^{-1}. For the first time, chromatins of different origins with increasing content of non-histone proteins have been investigated by Raman spectroscopy. The site of interaction of the non-histone proteins appears to involve the N7 position of guanine while the histone core does not interact at this site. It is proposed that the mechanism of specific recognition in chromatin involves the large groove.

INTRODUCTION

Considerable progress has been achieved recently in the understanding of the structure of chromatin by the discovery and isolation of repetitive subunits called nucleosomes[1-5]. These nucleosomes consist of a double hélical DNA of length about 200 base pairs wrapped around a protein core, made of four histones H2A, H2B, H3 and H4[6-9]. The results of recent studies on nucleosome crystals by X-ray diffraction and electron microscopy [10] are consistent with a model of flat particules of dimensions of 110 x 110 x 57 Å composed of the DNA double helix wound around a protein core as a flat superhelix having a pitch of about 28 Å [10]. However, more detailed information on the structure of both DNA and of protein core and on protein-DNA interactions are not yet available. Thus the precise structure and organization of DNA remains an open question. Several models have been proposed for the understanding of the DNA structure in chromatin :

i) bending of DNA around the histone core which will lead to a slight modification of the normal B form.

2) alternatively formation of more or less sharp kinks at periodic intervals[11-12].

3) structural changes leading to other forms such as the C or A forms[13].

Furthermore, little is known about the location of non-histone proteins which, although present in variable amounts according to the tissue, have an important functional role.

Vibrational spectra obtained by laser Raman scattering and also infrared spectroscopy have been shown to be a sensitive method for the determination of structural parameters of nucleic acids[14-19]. These vibrational spectra are composed of lines or bands whose frequencies and intensities depend on the nature of molecular groups, their geometry and environment. Recently, it was shown that the interaction of histones or protamine with DNA does not lead to a structural change B → A or B → C but in contrast the DNA B form is stabilized[21]. Similarly, the interaction of the DNA with the histones core prevents such structural transitions and DNA remains in a stable B-type form.

In the present work, Raman scattering has been used to study the structure of chromatin from a variety of sources as well as isolated intact mononucleosomes and nucleosomes involving two, three or four particles. In order to determine the structure of DNA in chromatins and in nucleosomes, the Raman spectra of the three major forms of natural DNA (A, B and C) have been characterized in fibres both by X-ray diffraction and Raman spectroscopy on the same sample. In addition, new information has been obtained indicating the site of interaction with DNA of chromosomal non-histone proteins (NHP).

RESULTS

Figure 1 (b) shows the Raman spectrum of an oriented fibre of Li-DNA (calf thymus) at 47 % relative humidity (R.H.). An X-ray diffraction pattern of the same specimen under identical conditions demonstrates that the fibre is in the C form. The Raman spectrum of the C form of DNA is characterized by a band at 865-870 cm^{-1}. The assignment of this spectrum to the C form of DNA has for the first time been confirmed by X-ray diffraction data obtained from the same specimen (Fig. 3b). Thus the band at around 865 cm^{-1} must be considered as being characteristic of the C form geometry, most probably being due to the phosphodiester stretching vibrations[22]. The Raman spectrum of the same DNA fibre but at 98 % relative humidity is shown in Figure 2 (a). It can be seen that the band at 865 cm^{-1} disappears while a band at 835 cm^{-1} appears. This band is characteristic of the B form of

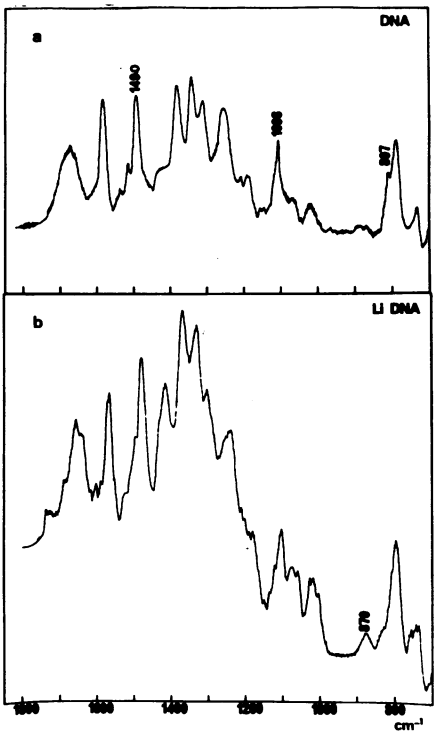

FIGURE 1 Raman spectra of DNA fibres in the A form (a) and C form (b). X-ray diffraction patterns from the same fibres are shown in Fig. 3 a and 3 b - (a) A spectrum from a fibre of Na-DNA at 75 % relative humidity (R.H.). The fibre was pulled from a 4 % solution of calf thymus DNA containing 3-4 % NaCl (w/w). The fibre exhibited a reversible A $\rightleftharpoons$B transition at higher humidity (98 % R.H.). - (b) A Raman spectrum from a fibre of Li-DNA ar 47 % R.H. The fibre was pulled from 8 % solution of calf thymus DNA dialyzed against LiCl solution at pH 7 to obtain about 3 % LiCl (w/w) content. The same fibre at high R.H. (98 %) yielded a B form X-ray diffraction pattern and the Raman spectrum shown in Fig. 2 a. The oriented DNA fibre was prepared following the method of Fuller et al.[37] and mounted in a Debye-Scherrer capillary tube for both Raman measurements and X-ray diffraction. A drop of saturated salt was placed to control the relative humidity and scaled. The capillary was thermostated at 15° C by means of a brass block sample holder. The fibre was allowed to reach equilibrium with constant humidity. Raman spectra were excited with the 514.5 nm line of an argon ion laser (Coherent Radiation CR.2) using about 150 mW to illuminate the fibre and about 500 mW for the solutions. Raman spectra were obtained on a Jarrell-Ash 25-400 spectrometer.

DNA and has been confirmed by X-ray diffraction analysis in good agreement with previous results of Peticolas et al.[15]. The above change is reversible with changes in the relative humidity.

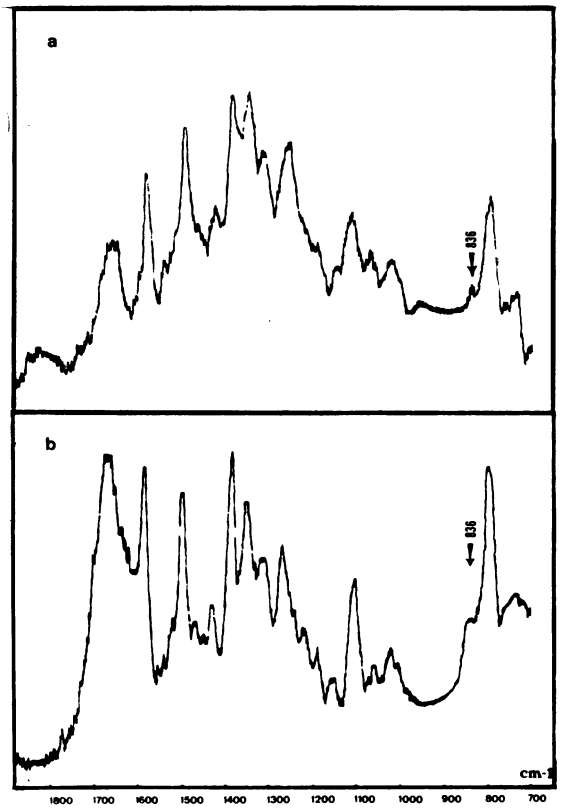

FIGURE 2 Raman spectra of the B form of DNA.
a) A spectrum obtained from the fibre of Li-DNA at 98 % R.H. which at lower R.H. (47 %) gave the spectrum shown in Fig. 1 b.
b) Spectrum taken from a 4 % solution of Na-DNA at pH 7.

Figure 1 (a) shows the Raman spectrum of an oriented fibre of Na-DNA at 75 % R.H. and which is characterized by a band at 807 cm^{-1}. An X-ray diffraction pattern of the same specimen is shown in Figure 3 (a) and can be seen to be of the classical A form. The band at 807 cm^{-1} has already been observed in vibrational spectra (Raman[15, 18] and I.R.[19]) of DNA and has been assigned to the single bond phosphodiester stretching vibration of the DNA backbone in the A geometry[18, 19 b&c]. At higher humidity (98 % R.H.) the fibre yielded a Raman spectrum exhibiting the 835 cm^{-1} band characteristic of the B form and which is also observed in solution (Fig. 2 b). Comparison of the Raman spectrum of the Li-DNA fibre in Figure 2 (a) with the solution spectrum (Fig. 2 b) indicates that they are essentially identical and that in both spectra the 835 cm^{-1} band of the B form is observable. Raman

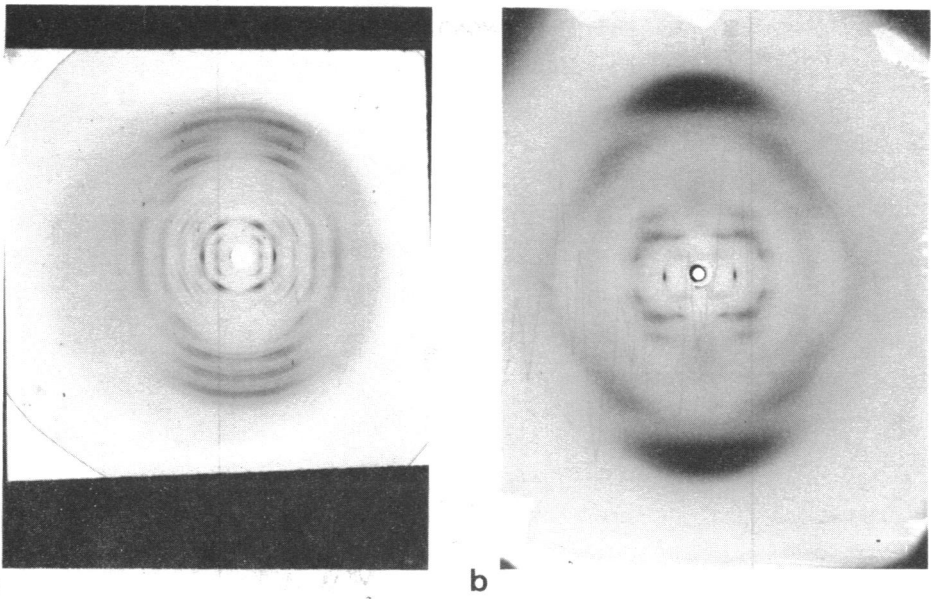

FIGURE 3 X-ray diffraction patterns of calf thymus DNA fibres from which Raman spectra have been measured as shown in Fig. 1.
a) Diffraction pattern of an oriented fibre of calf thymus Na-DNA with 3-4 % NaCl at 75 % R.H. showing the caracteristic features of the A form.
b) A diffraction pattern from the oriented fibre of calf thymus Li-DNA with about 3 % LiCl (w/w) at 47 % R.H. This is the characteristic diffraction pattern of the C form as evidenced by the appearence of an intensity peak around 0.1 A^{-1} on the first layer line and of "off meridional" intensity around 3.4 Å. Diffraction patterns were obtained using nickel filtered copper Kα radiation from an Elliott GX6 rotating anode generator. Samples were placed in a searle X-ray diffraction camera employing an Elliott toroidal focusing system.

spectroscopy enables one to determine qualitatively which of the conformations (A, B or C) is present in DNA samples in solution and fibres. The technique is not fully quantitative, however, only a small percentage (≤ 10 %)of a minor component could go undetected.

Raman spectra of mononucleosomes prepared from calf thymus are shown in Figure 4. The spectra are dominated by the Raman bands of DNA and one can clearly observe the band at about 1100 cm^{-1}, due to the phosphate semi-double bond O–P–O symmetric stretching vibration. Particularly important is the presence of a band at about 835 cm^{-1}- 840 cm^{-1} assigned to the phosphodiester stretching vibration in the geometry of the B form, as shown in the preceeding section. No bands characteristic of the C form

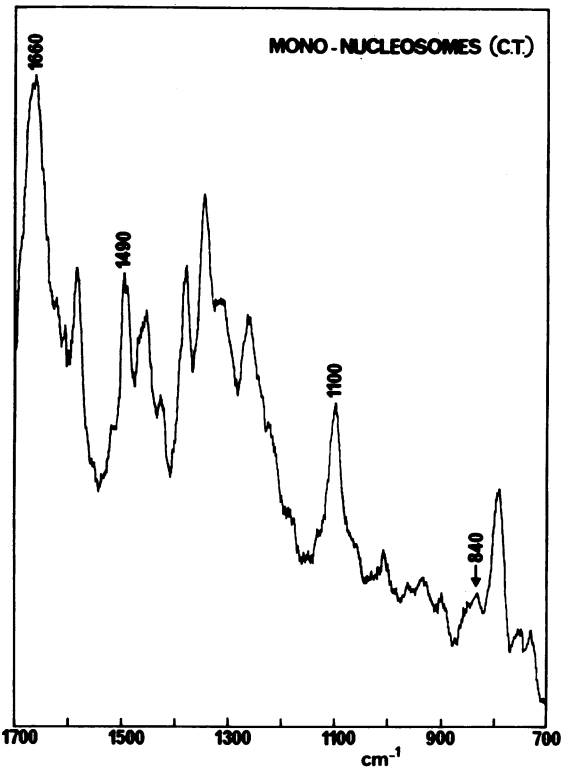

FIGURE 4 Raman spectrum obtained from a solution of mononucleosomes in 0.1 mM EDTA, 0.2 M phenylmethylsulfonylfluoride (PMSF) at pH 7.5, at 15° C. Nucleosomes were prepared by digestion of purified calf thymus nuclei with staphylococcal nuclease[38].

or A form can be detected. The correspondance of this 835 cm^{-1} band with the presented results on DNA Raman scattering and X-ray diffraction clearly rules out the A or C forms of DNA in nucleosomes.

Several bands characteristic of the base vibrations are present in the spectra of nucleosomes. The assignment of these bands was based on previous studies[15, 16]. Thus one can observe a band at 1490 cm^{-1} assigned predominantly to guanine stretching vibrations with a small contribution from adenine vibrations.

In addition to the vibrations of the DNA component the contribution of proteins can be clearly distinguished. This is reflected in the presence of bands at about 1260 cm^{-1} and at about 1660 cm^{-1}, i.e. at frequencies which correspond to Amide III and Amide I vibrational frequencies in the α helical conformation. The absence of any intense band

below 1240 cm^{-1} indicates that the β-pleated sheat conformation does not contribute significantly to the Raman spectra of nucleosomes.
The circular dichroism measurements of the nucleosomes also indicated that the α-helical structure is the predominant protein conformation in nucleosomes, representing about 50 % of the protein structure (manuscript in preparation). These results are in good agreement with the determination of Thomas et al.[23].

In order to determine the possibility of any changes in Raman spectra due to internucleosomal interactions and to tertiary or higher order structure di-, tri- and tetranucleosomes were investigated. The results of measurements on nucleosomes indicate that the band at 835 cm^{-1}-840 cm^{-1} is present in all nucleosomes particles studied which shows that the B or slightly modified B form is detected by Raman spectroscopy (Fig.5). There is no indication of changes of DNA structure in these nucleosomal structures of increasing number of particles, or of any influence of intra- or internucleosomal interactions in the nucleosomes studies. It has been suggested[10b] that the addition of 0.2 mM Mg^{2+} can cause chromatin to adopt a more compact structure. In order to determine whether any detectable changes in the nucleosome secondary structure is produced by Mg^{2+}, a Raman spectrum of trinucleosomes in 0.2 mM Mg^{2+} has been taken (not shown), but does not show any differences of band intensities of frequencies when compared to spectra obtained in the absence of magnesium. The absence of any observed effect with magnesium may be due to the lack of H1 in the nucleosome preparation since this appears to be necessary[10b], for the formation of the chromatin solenoidal structure. Alternatively, it may be that particles containing more than four nucleosomes are necessary for the change in tertiary structure to be apparent.

A Raman spectrum of chromatin from calf thymus is shown in Fig. 6. All the vibrational modes characteristic of DNA and of proteins in nucleosomes are present in the chromatin spectrum, but the intensity and position of some lines exhibit small changes. One can clearly observe the band at 835 cm^{-1} due to the presence of the B form geometry of the phosphodiester O-P-O single bond stretching vibrations as described in the preceeding section. This can be clearly seen by comparison with the spectra of DNA in the B form both in the fibres and in solution shown in Fig. 2. One can also observe the band at 1098 cm^{-1} assigned to O-P O semi-double bond stretching vibrations[22], and the bands which have been attributed to base vibrations. The protein conformation in chromatin from calf thymus has a large α-helical

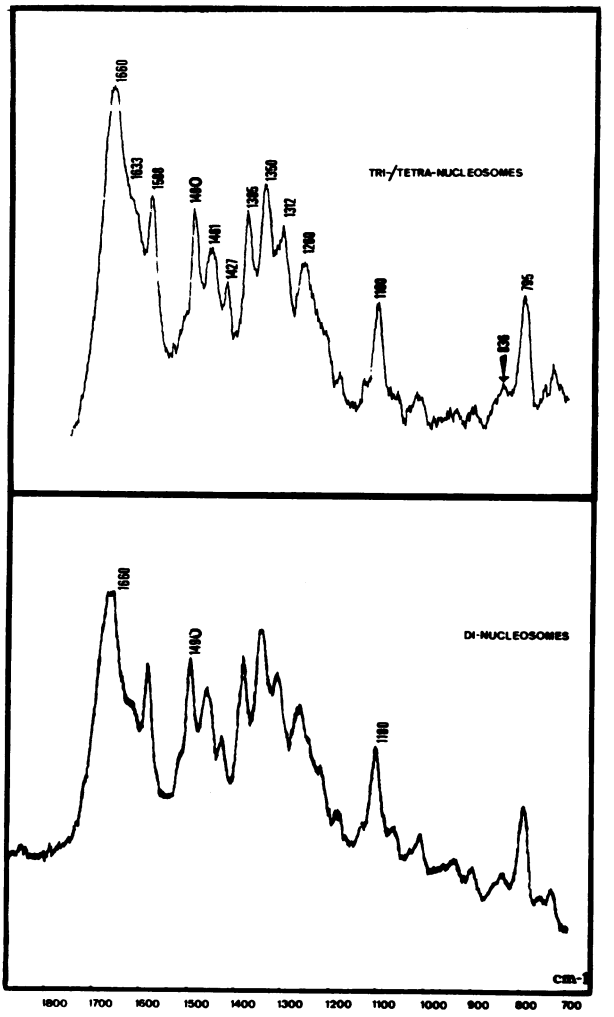

FIGURE 5 Raman spectra of dinucleosomes (above) and of tri- and tetra-nucleosomes in 0.1 M EDTA, 0.2 M PMSF at pH 7.5 (below) at 15° C. The nucleosomes particles were prepared by the method of Shaw [38], separated on an A5M Biogel column and identified by polyacrylamide gel electrophoresis. Concentration was between 0.6 % and 1 %.

component similar to that of nucleosomes as indicated by the frequency of Amide I at 1658 cm^{-1} (± 2 cm^{-1}) and Amide III bands.

The Raman spectra of chromatins from Zajdela hepatoma cells (Z.H.C.) and from the same hepatoma cell containing bromodeoxyuridine substituted DNA are shown in Fig. 7. The Raman bands of these chromatins are essentially similar to those of calf thymus but some changes in the intens-

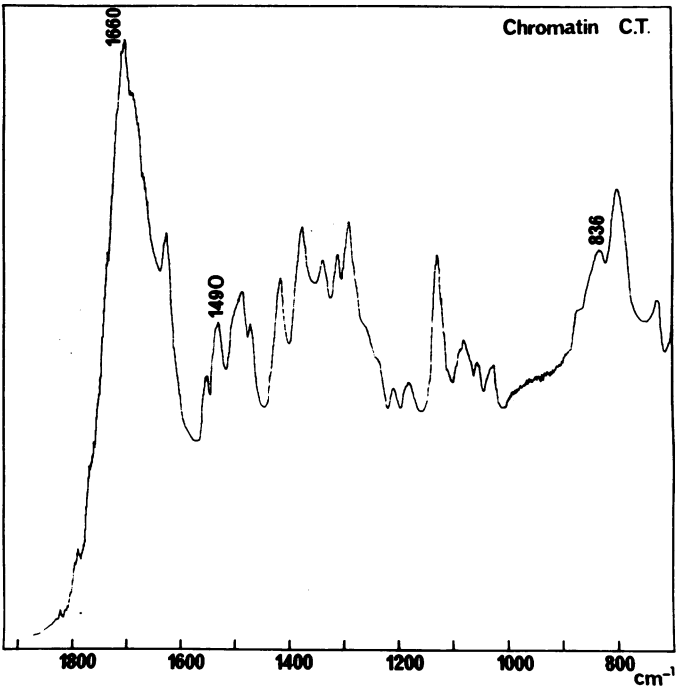

FIGURE 6 Raman spectrum of native chromatin from calf thymus in 0.2 mM NaCl, 0.2 mM EDTA, 0.2 mM PMSF, at pH 7.5 at 15° C. The chromatin was prepared according to the method of Reeder[39] modified by de Pomerai et al.[40]. This method uses only low ionic strength solutions, is based on nucrose centrifugation and avoids shearing. The Raman data are obtained at 15° C.

ities of particular bands are observed. In contrast to calf thymus chromatin, the chromatin from rat liver and Zajdela hepatoma cells are rich in non-histone proteins. The comparison of the Raman spectra of these chromatins leads to the following observations :

A/ The Raman lines due to Amide I and Amide III of histones are situated at 1658 cm^{-1} (± 2 cm^{-1}) and at about 1270 cm^{-1} respectively. Their position indicates a relatively high content of α-helical structure and a relatively small content of β structure.

B/ The band at about 1490 cm^{-1} is assigned predominantly to the guanine in plane vibration, involving N7-C8 stretching, with a small contribution from adenine. The comparison of the Raman spectra of N7 methyl guanine (Fig. 8 a) with that of GMP (Fig. 8 b) clearly indicates that the intensity of the 1490 cm^{-1} band is considerably reduced. It was also found

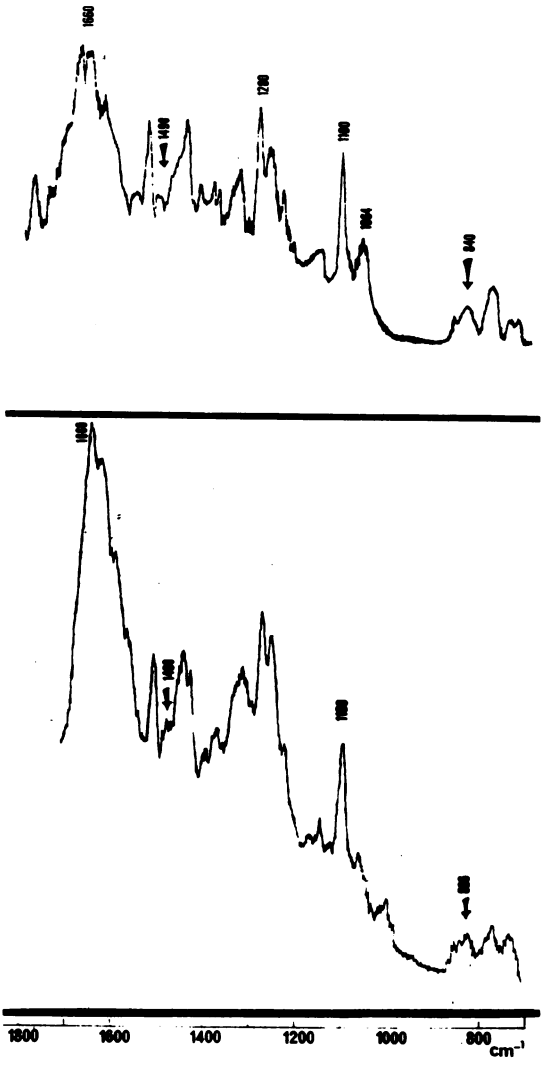

FIGURE 7 Raman spectra of chromatin extracted from Zajdela hepatoma cells. A spectrum (above) of chromatin containing 5-bromo-deoxyuridine substituted DNA is compared with a spectrum (below) of the native chromatin from Zajdela hepatoma cells. The method of preparation and the conditions under which Raman results were obtained are as for the spectrum in Fig. 6 and Fig. 1.

that in DNA upon progressive methylation at the N7 position of guanine, the intensity of this band decreases quantitatively to the level of 7-methyl guanosine.[41] That the changes in intensity of this 1490 cm^{-1} band should be correlated with the vibrations at N7 is also indicated by the observation that the protonation of N7 at pH 1 causes this band to

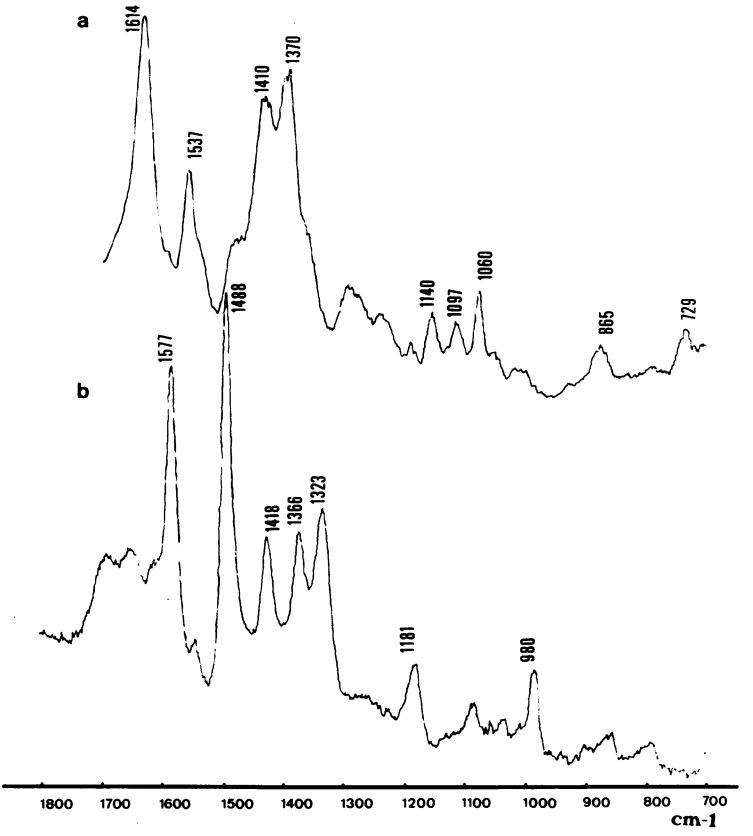

FIGURE 8 Raman spectra of 7N-methyl guanosine (a) and of GMP (b) in solution (6.0 %) at pH 6.8. The intense band at 1488 cm^{-1} in the spectrum of GMP is assigned to an in-plane vibration involving the guanine N7 position ; this band is not observed in the spectrum of 7 methyl guanine (see also[41].).

disappear[15]. This assignment is also in agreement with recent resonant Raman measurements, obtain in this laboratory, on 8-Br guanine. The substitution at the C-8 position by bromine causes a pronounced shift of about 17 cm^{-1} in this band[25].

Table I indicates that the relative intensity of the 1490 cm^{-1} band of guanine with respect to the phosphate band at about 1097 cm^{-1} taken as a standard decreases strongly in chromatins, as their content in non-histone proteins increases. In contrast, the relative intensity of the 1490 cm^{-1} band in nucleosomes (from calf thymus), which are devoid of NHP, is essentially identical to that in DNA.

TABLE I

RELATIVE INTENSITIES RATIO OF 1490 cm^{-1} BAND ($\pm$ 3 cm^{-1}) WITH RESPECT TO PHOSPHATE 1100 cm^{-1} BAND.

	1490 cm^{-1} (H_2O)
DNA	1.2
Mononucleosomes (Calf thymus)	1.2
Dinucleosomes (Calf thymus)	1.2
Tri-Tetranucleosomes (Calf thymus)	1.2
Chromatin Calf thymus	0.6
Chromatin Z. hepatoma cells	0.2
Chromatin Z. hepatoma cells BrdUrd Substit.	0.17

DISCUSSION

FORM OF DNA IN CHROMATIN. The results described here clearly demonstrate that the DNA component is in the B form or a slightly modified B conformation. This was unambiguously determined by Raman spectroscopy of chromatin preparations of various origins, of a series of nucleosomes and of DNA fibres and directly correlated with X-ray diffraction studies on the same DNA sample (see Fig. 1, 2, 3, 4 and 5). For this purpose the Raman spectra of the A and B forms of DNA have been characterized and are in good agreement with previous results of Peticolas et al.[15] whereas the C form of DNA is for the first time characterized by Raman scattering and X-ray diffraction. Hence the interaction of DNA with histones core and with NHP does not lead to any change to the A form or to the C form as has been suggested from model building studies[26] or from the interpretation of spectroscopic results (for a review see Fasman[13]). It is to be noted that our results on the DNA B form in chromatin and in nucleosomes are in good agreement with the explanation of the results of enzymatic digestion of chromatin[27, 8] and with early experiments on wide angle X-ray scattering of nucleohistones[28]. Our nucleosome studies have been performed in solution, however, neutron

scattering studies have shown that the structure in solution is also that found in crystals[27, 10a].

Since DNA is wrapped around the outside of the nucleosome[10] it must be in a modified B form (see "Introduction"). The extent to which the B form is modified depends upon the tertiary structure. If the DNA "superhelix" is composed of kinks[11] between which are straight segments of double helical structure, it is likely that such segments are of essentially unmodified B-DNA. The alternative hypothesis that the DNA is bent regularly into a smooth superhelix necessitates a modification of the B conformation and loss of helical symmetry. Such modifications will include, in the case of a smooth superhelix, a modest tilt of about 4°-5° of base planes with respect to helical axis. Our results do not allow us to differentiate between these two possibilities. The structure of the B or modified B form is particularly appropriate for the interaction with specific proteins like NHP, within its large groove (see below). In fact as calculated by Arnott[29], a modified B form has the largest width of its big groove (M = 1.35 nm) in which the NHP can be thus more easily located than in any other form like A form (M = 0.27 nm) or C form (M = 1.05 nm) of DNA.

PROTEIN INTERACTIONS. Our results on a series of nucleosomes and on chromatins from different origins have enabled us to draw some conclusions about the interactions and contacts between DNA and two major protein components of chromatins, the histone core and the non-histone proteins. These two components represent two kinds of protein-DNA interactions, specific and non-specific. For the first time chromatins od different origins with increasing content of NHP have been investigated by Raman spectroscopy. This includes chromatin from tissues not active in protein synthesis such as calf thymus, in which NHP represents only about 10 % by weight of the protein component, and also from very active tissues like liver or hepatoma cells in which the NHP equals the histone content. At the other extreme are the calf thymus nucleosomes which are devoid of NHP and of H1 in our preparations.

The results described here show that the Raman band at 1490 cm^{-1} assigned to vibrations involving the N7 position of guanine has the same relative intensity in DNA and in mono-, di-, tri- and tetranucleosomes from calf thymus. Thus the core histones cannot be involved in the interaction with the N7 position of guanine and are, therefore, not located in the large groove.

The reduction in the intensity of the 1490 cm^{-1} band is observed in

chromatins of various origins, and is more pronounced in chromatins having greater NHP content (see Table I). It is thus concluded that the NHP are directly involved in the interactions with N7 and are, hence, located in the DNA large groove. The variation in the intensity of the band at 1490 cm^{-1} with NHP content in chromatins possessing a similar complement of histones is further evidence that neither the histone core nor H1 interacts with the guanine N7. Hence, our comparative investigation indicates that the sites of interaction of the NHP and of histones are different and that the major groove is the site of NHP interactions.

The following features of the methods used in the present studies should be emphasised : 1) The method of Raman spectroscopy allows one to investigate the vibrations of particular groups, their three dimensional arrangement and their environment, in native chromatin. It does not necessitate the use of chemical probes which may modify the native structure or cause displacement or changes in the interacting groups or molecules in chromatin. - 2) We have carried out a comparative study on chromatins from different origins and on nucleosomes which has enabled us to obtain more complete structural information than recent Raman scattering studies which have been limited to chromatin from only one source[23-24] and from mono-nucleosomes[23].

The molecular mechanism of the recognition of specific base sequences of DNA by regulatory proteins represents one of the most important and unsolved problems in molecular biology. Several recent papers have tentatively proposed models for the recognition process involving protein interactions in the small groove of DNA[26a, 31-33]. These models postulate structural complementary between protein backbones in an antiparallel β-sheet conformation folded around the small groove. Other models have been proposed which consider the large groove as the site of interaction[34-36]. The proposed recognition in either case can only occur between the base atoms exposed in the DNA groove and amino-acid side chains. Up to now no experimental data supporting either model have been presented. In contrast, our Raman scattering results obtained from native chromatin indicate that the site of interaction and most probably of the recognition process in eukaryotes is the major groove and involves hydrogen bonding between the N7 group of guanine and the side-chain of NHP amino-acid residues. Six potential interacting sites in the large groove have to be considered including N7[34]. Work is in progress in our laboratory to obtain further information about the nature of the sites for specific and non-specific protein/DNA interactions.

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