LASER RAMAN SPECTRA OF CALF THYMUS CHROMATIN AND ITS CONSTITUENTS

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ABSTRACT Extensive Raman measurements have been made on calf thymus chromatin, core chromatin, the (H3.H4)/DNA complex, and isolated DNA. The results indicate that the α-helical content of the nucleosomal histones gradually increases as they form the heterocomplexes that lead to the formation of the octameric nucleosome core. The secondary structure of the latter is not modified as it binds to DNA. The spectra indicate that the DNA essentially retains its B conformation in nucleosomes, although slight changes probably occur in the ribose-phosphate backbone. No specific interactions between the nucleosomal histones and DNA can be established from the spectra, but histone H1 possibly interacts selectively with the thymine bases.

INTRODUCTION

It is now well established that the fundamental step of chromosomal DNA condensation involves the coiling of the double-stranded nucleic acid around a protein octamer consisting of two each of the histones H2A, H2B, H3, and H4 (for review see Igo-Kemenes et al., 1982; McGhee and Felsenfeld, 1980; Lilley and Pardon, 1979). The particles thus formed (core particles), which consist of one and three-fourths turns (145 base pairs) of DNA wrapped around the octameric protein core, have a flattened globular shape with height ~5.7 nm and diameter ~11.0 nm (Finch et al., 1981). Associated with these elementary subunits of chromatin is a fifth histone (H1 or H5), which is bound to the DNA at its point of entry and exit of the nucleosome as well as in the linker region (Boulikas et al., 1980).

Although there is general agreement about the external geometry of the polynucleosome chains in chromatin, much has still to be learned about the exact way in which histones and DNA interact in this system. Qualitatively, it appears that the inner histones interact intermolecularly through their globular regions (Böhm et al., 1977; Moss et al., 1976*a*, *b*) and recent Raman measurements on the H2A.H2B and H3.H4 complexes (Jutier et al., 1984) have confirmed the notion that electrostatic and hydrogen bonding interactions between charged acidic and basic residues play a major role in the formation and stability of histone/histone complexes. Similarly, the various models proposed so far for the binding of the histone octamer core to DNA are mostly on electrostatic interactions between the highly basic NH_2-terminal tail section of the histones and the phosphate groups of the nucleic acid.

In recent years, a new insight into the structure of chromatin and its constituents has been gained through the use of Raman spectroscopy. Thomas et al. (1977) recorded the Raman spectrum of the histone protein core of nucleosomes isolated from chicken and erythrocyte chromatin, and they showed that this histone complex in high-salt solutions contains a high percentage of α-helical structure (~50%), with no appreciable β-sheet conformation. The spectrum of whole chromatin from mouse myeloma and calf thymus was first obtained by Mansy et al. (1976), who noticed a marked decrease in the intensity of the 1,490 cm^{-1} guanine band in the complex, relative to free DNA. They attributed this effect to the formation of a hydrogen bond between the NH_2-terminal cationic residues of the histones and the N7 atom of the guanine bases in the major groove of the DNA, which was otherwise shown from the spectra to exist in its B form. The intensity change detected by these authors was not, however, observed by Thomas et al. (1977) in the spectrum of chromatin from chicken erythrocyte. The discrepancy between these results was later explained by Goodwin et al. (1978, 1979), who showed that the effect was attributable to the presence of nonhistone proteins (NHP) in the samples where the intensity change was noted. This suggested that the large groove of DNA provides a site of attachment for these proteins. These authors further concluded, from the large intensity increase of the 1,580 cm^{-1} band of adenine in the spectrum of nucleosomes stripped from their H1 content, that the core histones are involved in interactions with bases in the small groove of DNA.

The Raman spectra of individual histones extracted from calf thymus chromatin have been published (Guillot et al., 1977; Pézolet et al., 1980), and we have recently studied the H2A.H2B and H3.H4 complexes (Jutier et al., 1984). Here we report on the corresponding spectra of

chromatin, core chromatin, and the (H3.H4)/DNA complex. The method of spectral stripping has been used to better show the changes associated with each step of histone-DNA complexation in the formation of polynucleosomes.

MATERIALS AND METHODS

The chromatin was extracted from fresh calf thymus, using the method of Nadeau et al. (1974). Its various constituents were separated by chromatography on Bio-Gel A5m (100–200 mesh; Bio-Rad Laboratories, Richmond, CA), as described by Beaudette et al. (1981). In brief, H1 was separated from the chromatin by elution with a 0.6 M NaCl and 10 mM Tris buffer at pH 8, after dialysis against this same solution. The core chromatin thus obtained was concentrated by ultrafiltration over PM10 membranes (Amicon Corp., Scientific Sys. Div., Danvers, MA). The (H3.H4)/DNA complex was isolated from the core chromatin in a similar way, using a 1.25 M NaCl buffered solution. Finally, the DNA was separated from its histone complement by fractionation of either the core chromatin or the (H3.H4)/DNA complex in a 2.0 M NaCl-Tris buffer. The protein/DNA ratio in the isolated complexes was determined after each chromatographic separation from the ultraviolet (UV) spectrum of the collected samples (Raynauld and Ohlenbusch, 1972), to insure that the expected DNA/histone stoichiometry was in fact obtained.

For the Raman studies, the aqueous samples were concentrated by ultrafiltration over PM10 Amicon membranes (Amicon Corp., Scientific Sys. Div.). In general, highly concentrated gels were obtained (15–20% by weight, except for the [H3.H4]/DNA complex at 7% wt/wt). These gels were studied in capillary cells placed in a copper holder maintained at 10°C. The spectra were recorded on a microcomputer-controlled spectrometer (model 1400; Spex Industries, Inc., Metuchen, NJ) (Savoie et al., 1979), using the 514.5 nm exciting line from an argon ion laser (model 165; Spectra-Physics Inc., Mountain View, CA). The spectra of the samples were routinely measured through multiscan experiments to improve the signal-to-noise ratio. Signal averaging was commonly performed over 10 consecutive scans (4 h), yielding high-quality spectra, with an estimated intensity accuracy of ±3% for the major peaks. The corresponding spectra of the sample buffers were also obtained in each case for baseline subtraction.

RESULTS

Raman spectra were obtained from at least three complete series of samples from chromatin and its various constituents; the best results are shown in Fig. 1. All of these spectra, as well as those shown in the other figures, are solvent-subtracted spectra. For the subtraction, the spectrum of the solvent (but not that of the sample) was smoothed over 21 data points by the procedure of Savitzky and Golay (1964), except for the regions where sharp peaks occurred (e.g., the region of the cacodylate peak at 610 cm^{-1}). To establish the relative intensity of the solvent peaks in both spectra, we have used the method of Williams and Dunker (1981), which is based on the relative intensity of the water stretching band in the 2,500–3,100 cm^{-1} region. This method was believed to be more reliable than that based on the use of sharp marker bands, such as the 610 cm^{-1} peak of the cacodylate ion, since the height of such sharp peaks can easily be affected by wavelength tracking errors in multiscan experiments. For a better presentation of the results, the luminescent background present in some of the spectra was routinely removed by computer subtraction of an appropriate third degree polynomial function from the original trace.

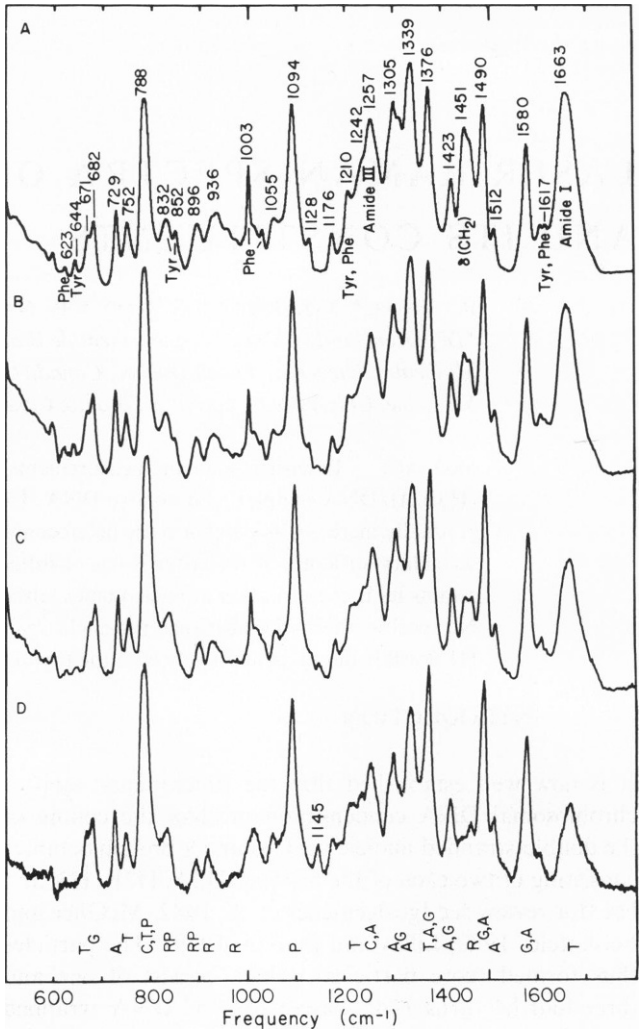

FIGURE 1 Solvent-subtracted Raman spectra of calf thymus chromatin constituents are shown: (*A*) chromatin (15% wt/wt, H_2O); (*B*) core chromatin (15% wt/wt, 0.6 M NaCl); (*C*) the (H3.H4)/DNA complex (10% wt/wt, 1.25 M NaCl); (*D*) DNA (15% wt/wt, 2 M NaCl). Experimental conditions are the following: laser power at sample, 300 mW; spectral slit width, 5 cm^{-1}; integration time, 18 s (9 scans at 2 s per data point); frequency increment, 2 cm^{-1}; temperature 10°C.

The series of spectra shown in Fig. 1 are dominated by the characteristic bands of the DNA bases. Note that the height of the 1,490 cm^{-1} peak relative to the phosphate band at 1,094 cm^{-1} in the spectrum of chromatin (Fig. 1 *A*) is very nearly equal to 1.0. This result contrasts with the value of 0.8 obtained by Mansy et al. (1976) and that of 0.6 measured by Goodwin et al. (1979). These last authors have shown that a low value for this ratio is indicative of the presence of nonhistone proteins in the sample. We therefore conclude that the method we have used for the preparation of chromatin has yielded a product that was relatively free of NHP. The protein/DNA ratio deduced from UV absorbance measurements (Raynauld and Ohlenbusch, 1972) also confirmed this

point. Therefore, the term chromatin used here is practically equivalent to polynucleosomes, and core chromatin refers to polynucleosomes stripped of H1.

A comparison of the spectra in Fig. 1 clearly shows the increased contribution of the protein bands as one goes from DNA to chromatin. The effect is particularly pronounced in the amide I and amide III regions, as well as in the $\delta(CH_2)$ region at 1,450 cm^{-1}. Also seen in the spectra are sharp bands from the protein aromatic residues, such as the 1,003 cm^{-1} peak of phenylalanine and the 852 cm^{-1} component of the tyrosine doublet.

In an attempt to specify the contribution of DNA and the various protein subunits to the spectra of complexes studied we have used the method of spectral stripping, using certain bands of DNA such as that at 1,094 cm^{-1}, which are known to be particularly insensible to various external effects, and the 1,450 cm^{-1} band of the proteins as intensity standards (assuming that the latter is directly proportional to the number of CH_2 groups in any one histone). To illustrate how this method works, let's consider the spectral contribution of the H1 moiety in chromatin (H1*; hereafter a star will be used to designate a particular subunit within a complex). Formally: chromatin = H1* + core chromatin* or H1* = chromatin − core chromatin*. Since the spectral contribution of the core chromatin in chromatin (core chromatin*) is not directly available, the experimental spectrum of core chromatin has to be used instead: H1* = chromatin − core chromatin. Obviously, if the spectrum of the core chromatin is modified by the presence of H1 in chromatin, this will cause extraneous features to be present in the difference spectrum (H1*), and these will have to be interpreted accordingly. Another factor to be considered is the scaling of the spectrum to be subtracted relative to that of the complex: H1* = chromatin − A × (core chromatin*), where the multiplier (A) has to be chosen so as to eliminate from the difference spectrum the band present at 1,094 cm^{-1} in both starting spectra. The bands at 788 and 1,580 cm^{-1} can also be used in this case, as they are of comparable intensity (relative to the peak at 1,094 cm^{-1}) in the spectra of chromatin and core chromatin. The values of the scaling factory (A) for the spectral subtractions presented here are probably accurate to ±5% and a variation of this magnitude does not affect the difference spectra to the point where it could modify the interpretation of the results.

The equivalent spectrum of H1* obtained in the above manner is reproduced in Fig. 2, together with the experimental spectrum of an aqueous solution of histone H1 isolated from calf thymus. Many of the weaker peaks in H1* are derivative features resulting from a lack of exact frequency coincidence between the spectra of chromatin and core chromatin. For example, the apparent weak peak at 1,495 cm^{-1} in H1*, which is absent from the spectrum of H1, is in fact a component of a derivative feature with corresponding minimum at 1,487 cm^{-1}, resulting from a

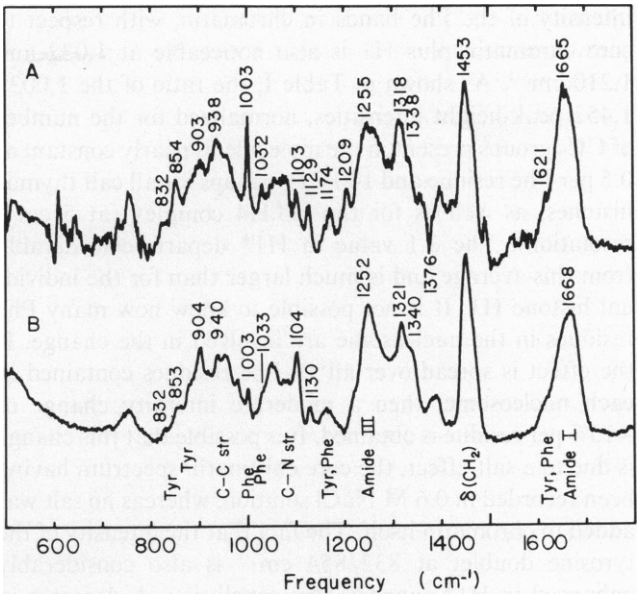

FIGURE 2 Solvent-subtracted Raman spectra of calf thymus histone H1 are shown: (A) difference spectrum H1* = (chromatin) − (core chromatin) and (B) experimental spectrum from an aqueous solution of H1 (4% wt/wt). Experimental conditions for B are the following: laser power at sample, 100 mW; spectral slit width, 5 cm^{-1}; integration time, 20 s (10 scans at 2 s per data point); frequency increment, 2 cm^{-1}; temperature, 9°C.

slight shift of the 1,490 cm^{-1} band of DNA. Although this shift could be real and meaningful, the wavelength tracking accuracy of our instrument is not sufficient to verify this point. One also notes in H1* intensity differences involving some of the DNA bands. Such is the case with the negative peak at 1,376 cm^{-1} and the increased intensity of the 1,665 cm^{-1} band with respect to H1. These changes will be discussed below in the section dealing with DNA*.

One of the most striking features in H1* is the very strong phenylalanine (Phe) band at 1,003 cm^{-1}, which is about four times as intense as in H1. This increase in

TABLE I
INTENSITY RATIO OF THE 1,003/1,450 cm^{-1} BANDS NORMALIZED TO 1 Phe RESIDUE AND 100 CH_2 GROUPS IN THE RAMAN SPECTRA OF CALF THYMUS HISTONES AND THEIR COMPLEXES

Histone	Number of Phe residues	Intensity ratio
H2A	1	0.56
H2B	2	0.49
H3	4	0.49
H4	2	0.46
H3/H4	6	0.49
H1	1	0.53
H1*	1	2.14
Octamer*	18	0.52
Total histones*	19	0.57

H1* = [chromatin] − [core chromatin]; octamer* = [core chromatin] − [DNA]; Total histones* = [chromatin] − [DNA].

intensity of the Phe bands in chromatin, with respect to core chromatin plus H1 is also noticeable at 1,032 and 1,210 cm^{-1}. As shown in Table I, the ratio of the 1,003/1,452 peak-height intensities, normalized for the number of CH_2 groups present in the molecule, is nearly constant at 0.5 per Phe residue and 100 CH_2 groups for all calf thymus histones, as well as for the H3.H4 complex (at 5 cm^{-1} resolution). The 2.1 value in H1* departs considerably from this average and is much larger than for the individual histone H1. It is not possible to know how many Phe residues in the nucleosome are involved in the change. If the effect is spread over all 19 Phe residues contained in each nucleosome, then a moderate intensity change of ~15% per residue is obtained. It is possible that this change is due to a salt effect, the core chromatin spectrum having been recorded in 0.6 M NaCl solution, whereas no salt was added to chromatin itself. The fact that the intensity of the tyrosine doublet at 832/854 cm^{-1} is also considerably enhanced in H1* supports this conclusion. A decrease in intensity of the 1,003 cm^{-1} band of Phe in the Raman spectra of certain types of viruses upon increasing the ionic strength of the solutions has also been reported by Thomas et al. (1983). The obvious interpretation for this effect is a change in the stacking interactions of the protein aromatic residues. However, it is unlikely that these interactions involve the DNA bases as the Phe residues are probably buried within the globular part of the histones in both the core octamer and H1.

Using the method described above, it is possible to evaluate the contribution of the $(H2A.H2B.H3.H4)_2$ histone core to the Raman spectrum of core chromatin by subtraction of the spectrum of DNA: octamer* = core chromatin − DNA. The resulting spectrum is shown in Fig. 3 *A*. In the present study, due to the method of extraction used, we were not able to concentrate enough on the octameric core protein so as to obtain a satisfactory Raman spectrum of this complex. The spectrum reproduced in Fig. 3 *B* is a digitized version of that published by Thomas et al. (1977) after an approximate subtraction of a water spectrum. Note that this spectrum was obtained from chicken erythrocyte histones, but the comparison should be valid as the primary structure of these histones should differ little from that of the corresponding calf thymus histones (Von Holt et al., 1979). Note also that some dissociation of the octamer (referred to as a "heterotypic tetramer" by Thomas et al. [1977]) can occur (Godfrey et al., 1980), but the amount of this dissociation should be very small at the concentrations used in Raman spectroscopy.

Even though the difference spectrum shown in Fig. 3 *A* was generated from spectra recorded at 5 cm^{-1} slit width and the experimental spectrum (Fig. 3 *B*) was obtained experimentally at 10 cm^{-1} slits, these spectra are very similar particularly in the amide I and amide III regions. This indicates that the overall structure of the protein core in the nucleosome differs very little from that of the

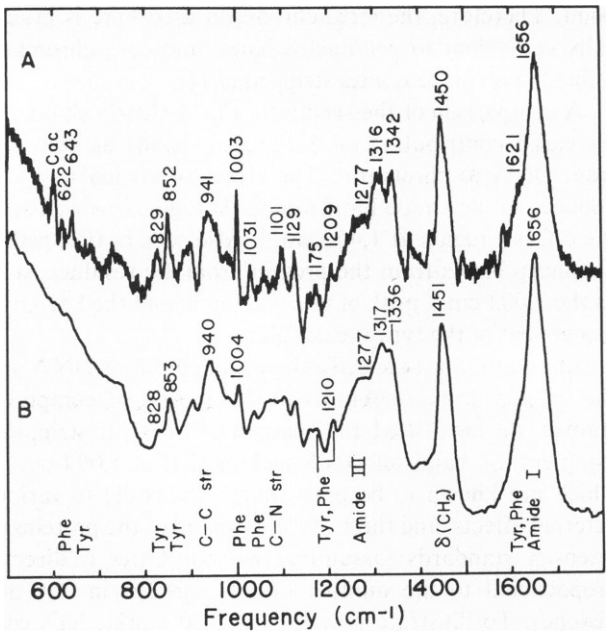

FIGURE 3 Solvent-subtracted Raman spectra of the nucleosome octameric protein core $(H2A.H2B.H3.H4)_2$ are shown: (*A*) difference spectrum Oct* = (core chromatin) − (DNA) (from calf thymus) and (*B*) digitized version of the experimental spectrum obtained by Thomas et al. (1977) (chicken erythrocyte). Experimental conditions for *B* are the following: laser power at sample (488.0 nm), 300 mW; spectral slit width, 10 cm^{-1}; temperature, 32°C; concentration, 9% wt/wt, 2 M NaCl.

isolated complex in 2 M NaCl solution. Also all of the DNA bands are reasonably well canceled off in the spectrum of the octamer*, which shows that the spectrum of DNA is little modified by the presence of the inner histones in the core chromatin, as would be expected if the protein-DNA interactions were mainly of the electrostatic type. Finally, an apparent increase in the intensity of the Phe bands, particularly at 1,003 cm^{-1}, is obvious in Fig. 3 *A*. However, when the intensity ratio of this peak with respect to that at 1,452 cm^{-1} is considered, a normal value of 0.52 is obtained (Table I). As in the case of H1*, we believe that the explanation has to do with the ionic strength of the various solutions involved. The DNA bound octamer* spectrum originates from that of core chromatin (0.6 M NaCl), whereas that of the DNA free octamer was obtained in 2 M NaCl solution.

It is now well established that histones H3 and H4 can form nucleosome like particles with DNA. It has been shown (Thomas and Oudet, 1979) that these particles, which have roughly the same overall dimension, can contain either a tetrameric $(H3.H4)_2$ or octameric $(H3.H4)_4$ core. Similarly, in polynucleosomes stripped of their H1 and H2A.H2B content, some of the $(H3.H4)_2$ tetramers can migrate to other stripped cores, leaving free DNA and forming octameric complexes (Stockley and Thomas, 1979). In the present study, the complex obtained had a protein/DNA ratio of 0.4 (wt/wt), which is appropriate for one $(H3.H4)_2$ tetramer per 200 base pairs of DNA.

However, it was not possible to determine the exact nature of this complex or even to ascertain that only one type of complex was present. Therefore, the term (H3.H4)/DNA complex used below can refer to either the tetrameric or the octameric complex and even to the dead-end $(H3.H4)_2$/DNA complex recently reported by Daban and Cantor (1982).

The (H3.H4)/DNA complex, isolated directly from core chromatin in 1.25 M NaCl in the present study, gave Raman spectra (Fig. 1 *C*) of lesser quality than chromatin and core chromatin. This, added to the relatively lower protein content of the complex, led to very noisy difference spectra: (H3.H4)* = (H3.H4)/DNA − DNA. An example of such a spectrum is compared in Fig. 4 to the spectrum of an aqueous solution of the H3.H4 complex (believed to exist as a $[H3.H4]_2$ tetramer) (Jutier et al., 1984). Despite the very poor quality of the difference spectrum, some changes were noted in all three series of measurements made on this system and they are believed to be real. Of particular interest are the intensity increase at 1,053 cm^{-1}, which could be associated with a perturbation of the DNA backbone in the complex, and the negative intensity difference at 1,250 cm^{-1}, which indicates that the α-helical content of the H3.H4 complex increases appreciably upon complexation with DNA.

The effect of H1 on the structure of chromatin can be evaluated through the difference spectrum core chromatin* = chromatin − H1. The results are shown in Fig. 5. Note that to better compare the spectra in this figure, portions of one of the spectra were progressively raised (or lowered) by computer addition (or subtraction) of a linear function, so as to have common base points in the two spectra. Some of the changes observed here, such as the enhancement of the Phe bands at 1,003 and 1,209 cm^{-1}, are equivalent to those detected in the complementary H1* spectrum. As for the DNA bands, it is clear from Fig. 5 that they are not generally influenced by the presence of H1 in the chromatin complex. The small change in intensity of the 1,376 cm^{-1} band could be of some significance, as this band of mixed origin (thymine, adenine, and guanine) has also been observed to decrease in intensity (by 40%) in nucleosomes from rat liver, as compared with free DNA (Goodwin et al., 1979). However, the present observation cannot be considered as very meaningful, as the intensity variation noted in the particular spectra shown in Fig. 5 was not reproduced in other series of measurements. In the 1,663 cm^{-1} region, an intensity increase was generally observed in core chromatin* with respect to core chromatin itself. However, the magnitude of this change (~7% relative intensity, on the average) is comparable with the standard deviation on the measured values, and the effect is only believed to be meaningful in that it parallels a similar observation made by Chinsky and Turpin (1982) on the H1-poly(dA–dT) complex. The intensity change upon complex formation (~100%) was, however, much larger in that case due to the shorter wavelength of the exciting light (300 nm).

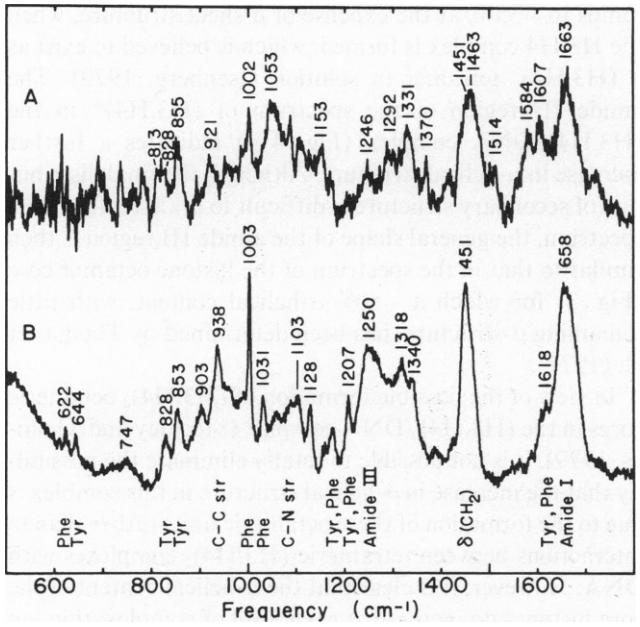

FIGURE 4 Solvent-subtracted Raman spectra of calf thymus H3.H4 complex are shown: (*A*) difference spectrum (H3.H4)* = (H3.H4)/DNA − DNA and (*B*) experimental spectrum from an aqueous solution (7% wt/wt). Experimental conditions for *B* are the following: laser power at sample, 250 mW; spectral slit width, 5 cm^{-1}; integration time, 14 s (7 scans at 2 s per data point); frequency increment, 2 cm^{-1}; temperature, 10°C.

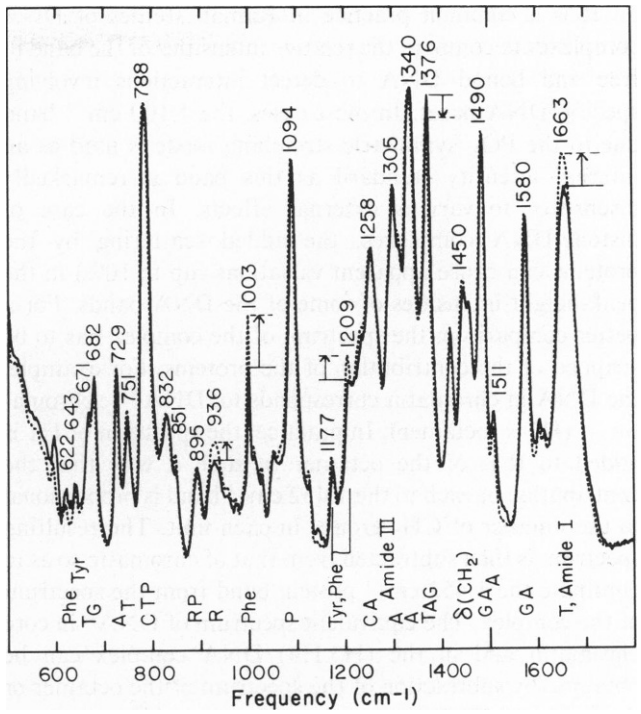

FIGURE 5 Solvent-subtracted Raman spectra of core chromatin from calf thymus are shown: (—) experimental spectrum (experimental conditions given in Fig. 1) and (· · ·) difference spectrum; core chromatin* = chromatin − H1.

It is a common practice in Raman studies of DNA complexes to compare the relative intensities of the band of free and bound DNA to detect interactions involving specific DNA bases. In most cases, the 1,100 cm^{-1} band due to the PO_2^- symmetric stretching mode is used as an internal intensity standard as this band is remarkedly insensitive to various external effects. In the case of histone-DNA complexes, the added scattering by the proteins can cause apparent variations (up to 10%) in the peak-height intensities of some of the DNA bands. For a better comparison, the spectrum of the complex has to be stripped of the contribution of the proteins. For example, the DNA in chromatin corresponds to: DNA* = chromatin − (H1 + octamer). In practice, the spectrum of H1 is added to that of the octamer in such a way that the contribution of each to the 1,452 cm^{-1} band is proportional to the number of CH_2 groups in each unit. The resulting spectrum is then subtracted from that of chromatin so as to eliminate the 1,452 cm^{-1} protein band from the spectrum of the complex. The equivalent spectrum of DNA* in core chromatin and in the (H3.H4)/DNA complex can be obtained by subtraction of the spectrum of the octamer or that of the (H3.H4) complex. The resulting spectra of DNA* can be directly compared with that of DNA, using the 1,094 cm^{-1} phosphate band (with baseline points at 1,038 and 1,162 cm^{-1}) as intensity standard. In the present study, although the relative intensities of the main DNA* peaks thus determined are believed to be accurate to ±3%, we have observed some small systematic variations between different complete series of measurements. The values reported below are therefore averages.

The above procedure applied to the various complexes discussed here indicates that the intensities of the DNA bands are not appreciably modified following the formation of polynucleosomes. Among some of the bands of interest, that at 1,490 cm^{-1} (baseline points at 1,405 and 1,545 cm^{-1}) is found to have a constant relative intensity (1.03 ± 0.03) in DNA* from chromatin, core chromatin, and the (H3.H4)/DNA complex, as well as in free DNA. This band, due to G and A bases, was found to have a relative intensity of 0.6 in calf thymus chromatin (Goodwin et al., 1979), this low value being attributed to the presence of nonhistone proteins in the samples. Similarly, the relative intensity of the DNA band at 1,580 cm^{-1} (baseline points at 1,545 and 1,740 cm^{-1}), assigned to guanine and adenine ring modes, shows little variation (it falls in the 0.85–0.81 range) in the spectra of the complex and in DNA. Our results in this case are at variance with those of Goodwin et al. (1979), who found this band to have a much higher relative intensity in calf thymus DNA (1.3) than in polynucleosomes of the same origin (0.8). Note, however, that these results were obtained in ^2H_2O solutions. Even so, other measurements on DNA in ^2H_2O, such as those of Erfurth and Peticolas (1975*b*), indicate that the 1,580 cm^{-1} peak has approximately the same intensity as the 1,094 cm^{-1} reference band.

The bands at 788, 1,580, and 1,663 cm^{-1} were found to have their intensity decreased by ~5% in DNA* from core chromatin, when compared with free DNA. As the observed changes are at the limit of the combined uncertainty on the measurements, this cannot be considered as meaningful at this point. Nevertheless, note that as the band involved are known to display a strong Raman hypochromism effect in DNA (Small and Peticolas, 1971; Erfurth and Peticolas, 1975*b*), a reduction in their intensity could indicate reduced stacking interactions of the DNA bases in chromatin and core chromatin. Such an effect was reported by Prescott et al. (1976) with polylysine bound to DNA.

DISCUSSION

The method of spectral stripping used here allows for the comparison of the spectra of the individual components of polynucleosomes with the corresponding spectral contribution of these same units in the various complexes involved. It is therefore possible to use these results to specify the structural changes occurring at each level of the nucleosome assembly.

Inner Histones

Our previous Raman study on the H2A.H2B and H3.H4 complexes (Jutier et al., 1984) has shown that the complexation of the individual histones led to an increase in α-helical structure. The effect is particularly important in the case of H3 and H4, whose ~20% α-helical content jumps to ~35%, at the expense of β-sheet structure, when the H3.H4 complex is formed, which is believed to exist as a $(H3.H4)_2$ tetramer in solution (Isenberg, 1979). The amide III region of the spectrum of (H3.H4)* in the (H3.H4)/DNA complex (Fig. 4 *A*) indicates a further increase in α-helical structure. Although the final distribution of secondary structure is difficult to evaluate from this spectrum, the general shape of the amide III region is then similar to that in the spectrum of the histone octamer core (Fig. 3) for which a ~50% α-helical content, with little remaining β-structure, has been determined by Thomas et al. (1977).

In view of the possible formation of $(H3.H4)_4$ octameric cores in the (H3.H4)/DNA complex (Stockley and Thomas, 1979), it is not possible to totally eliminate the possibility that the increase in α-helical structure in this complex is due to the formation of these octameric units rather than to interactions between tetrameric $(H3.H4)_2$ complexes with DNA. However, it is clear that the α-helical content of the core histones do increase at each level of complexation, up to the formation of the $(H2A.H2B.H3.H4)_2$ octamer. The generation of α-helical structure through complexation of the inner histones, which appears to be a critical step in the reconstitution of good histone-DNA complexes (Yu et al., 1976), is clearly followed in the Raman spectra of the various subunits of the nucleosome core. On the other

hand, the present results indicate that once the core octamer is formed its structure is not appreciably modified by the presence of DNA in the nucleosome, as evidenced by the similarity of the spectra shown in Fig. 3.

It is generally believed that the structured regions of the inner histones are located in the central globular regions of these proteins, where the histone-histone interactions mainly take place. The lack of any significant change in the secondary structure of the histone octameric core on nucleosome formation is therefore consistent with the notion that the DNA-histone interactions occur on the outside of the globular portion of the octamer core. That the inner part of this core is not appreciably perturbed on formation of the nucleosome is also suggested from the constant intensity ratio of the components of the tyrosine doublet at 850/830 cm^{-1} (Siamwiza et al., 1975) in the spectra of the histone core (Fig. 3).

Histone H1

Although the amide III band at 1,253 cm^{-1} in the H1* spectrum (Fig. 2) is somewhat broader than in H1, the similarity in the intensity distribution in both cases confirms the general belief that the secondary structure of this histone, as well as those of the inner histones and DNA, is little modified in chromatin. This histone is not required for the integrity of the chromatin subunit, even though there is some evidence that it is spread over most of the nucleosome (Belyavsky et al., 1980) and that it can interact with the inner histone core (Boulikas et al., 1980). The Raman spectra also indicate that the structure of histone H1 associated with DNA in polynucleosomes is appreciably disordered as is the case for the isolated histone in aqueous solution (Guillot et al., 1977).

DNA

It has already been established from previous Raman studies on chromatin (Mansy et al., 1976; Thomas et al., 1977; Goodwin et al., 1978, 1979) that the B-form conformation of DNA is retained on nucleosome formation. This is confirmed in the present study, the relative intensity of the 832 cm^{-1} band, due to the backbone phosphodiester stretching mode (Erfurth et al., 1972, 1975*a*; Prescott et al., 1984), is the same (0.42) in DNA* from chromatin and core chromatin as well as in free DNA. There are, however, indications in the spectra that some distortion of the deoxyribose-phosphate backbone of DNA does take place in nucleosomes. It has already been noted by Thomas et al. (1977) that weak Raman lines at 1,015 and 1,145 cm^{-1} are affected by the association with the inner histones. As can be seen in Fig. 1, the band at 1,146 cm^{-1} in the spectrum of DNA looses most of its intensity in core chromatin, whereas the intensity of the 1,015 cm^{-1} band is considerably reduced. These two bands, which are present in the spectra of ribose and deoxyribose (Small and Peticolas, 1971), have been attributed to ribose backbone vibrations in B-DNA (Lu et al., 1977). It is, therefore, likely that the structural modifications responsible for the above-mentioned spectral changes involve a distortion of the deoxyribose units or a change in the relative orientation of these units. A small change in the orientations of the phosphate groups of B-DNA with respect to the helical axis has been detected by infrared linear dichroism on complexation with poly(L-arginine) and poly(L-lysine) (Liquier et al., 1975). Most likely, this is the sort of small rearrangement that takes place on nucleosome formation. It is also interesting to note that the intensity changes detected here at 1,015 and 1,146 cm^{-1} are identical to those observed at 1,044 and 1,158 cm^{-1} in the spectrum of t-RNA on thermal disordering (Small et al., 1972).

The Raman spectra suggest that the DNA structure is also modified in the (H3.H4)/DNA complex. The bands at 1,015 and 1,146 cm^{-1} have intensities that are intermediate between those of free DNA and core chromatin, indicating that the change in the DNA backbone conformation associated with nucleosome formation also partly takes place in the (H3.H4)/DNA complex. Moreover, the difference spectrum (H3.H4)* (Fig. 4) shows an intense positive feature at 1,055 cm^{-1}, which coincides with a ribose-backbone vibration of B-DNA (Lu et al., 1977). This difference band, which is not present in the octamer* spectrum (Fig. 3), possibly reflects an even greater distortion of DNA than in the nucleosome. Note that a distorted shape for the (H3.H4)/DNA nucleosomelike particle is expected, in view of the anomalies found in the low-angle diffraction pattern of these particles (Boseley et al., 1976).

It is not possible to ascertain from our Raman measurements the existence of specific interactions between the inner histones and DNA bases in nucleosomes. It was concluded in an earlier Raman study (Goodwin et al., 1978, 1979) that the core histones interact with the nucleic bases, particularly adenine, in the small groove of DNA. This conclusion was based on an observed decrease in intensity of the 1,580 cm^{-1} band of DNA in nucleosomes, an effect that is not substantiated by the present study. On the other hand, the possibility of specific interactions between H1 and the C=O groups of the thymine bases cannot be discarded from the present results. The uncertain intensity decrease of the 1,663 cm^{-1} thymine band in the spectrum of chromatin vs. core chromatin could be related to the much larger change detected by resonance Raman spectroscopy in the H1-poly(dA–dT) complex (Chinsky and Turpin, 1982). Histone H1, with its highly basic (40% Lys) COOH-terminal half, is known to interact preferentially with nucleic acids rich in adenine-thymine bases (Sponar and Sormova, 1972). Whether or not these interactions can be detected by conventional Raman spectroscopy is questionable, and the small intensity change noted here would have to be independently confirmed to be considered meaningful.

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