Nucleosome Structure: Sites of Interaction of Proteins in the DNA Grooves as Determined by Raman Scattering†

D. C. Goodwin, J. Vergne, J. Brahms,* N. Defer, and J. Kruh‡

ABSTRACT: The purpose of this work is to localize histone and nonhistone proteins (NHP) in the DNA grooves. In order to localize the NHP we have compared nucleosomes from different sources which differ in their NHP content. A reduction in the intensity of the band at 1490 cm^{-1} has been observed, and a correlation between the magnitude of the reduction and the NHP content of the nucleosome preparation has been found. In order to localize histones we have compared nucleosomes with free DNA. We have found a decrease in the intensity of the Raman band at around 1580 cm^{-1}. The 1490- and 1580-cm^{-1} Raman bands in DNA result predominantly from guanine and adenine vibrational modes, respectively. As an aid to the interpretation of the data, Raman spectra have been taken from solutions of methylated forms of certain bases and from calf thymus DNA methylated with dimethyl sulfate which is known to methylate DNA at the guanine N(7) position and at the adenine N(3) position [Lawley, P. D., & Brookes, P. (1963) *Biochem. J. 89*, 127–138; Maxam, A. M., & Gilbert, W. (1977) *Proc. Natl. Acad. Sci. U.S.A. 74*, 560–564]. A decrease in the intensities of the 1490- and 1580-cm^{-1} bands has been observed upon DNA methylation. The data are consistent with a model for nucleosomes in which the large groove is accessible as a site of attachment of nonhistone proteins (NHP), while the core histones are involved in interactions with bases in the small groove.

Although considerable progress has been achieved with regard to the chromatin structure by the discovery of repetitive subunits (nucleosomes), their detailed structure and the mechanism of gene expression and of its regulation are not yet known (for review, see Elgin & Weintraub, 1975; Van Holde & Isenberg, 1975; Kornberg, 1977; Felsenfeld, 1978). Neutron scattering, X-ray diffraction, and electron microscopy of nucleosomes are in agreement with a model of flat particles in which DNA is wrapped around the histone core of dimensions 110 × 110 × 57 Å (Pardon et al., 1977; Carpenter et al., 1976; Finch et al., 1977; Oudet et al., 1975; Ollins et al., 1974). It was found that the DNA is in a B-like form (Goodwin & Brahms, 1978). It is of particular importance, both structurally and functionally, to determine the sites and mode of interaction of the two major types of proteins associated with chromatin, core histones, and nonhistone proteins (NHP[1]). Tentative models have been proposed for the binding site of histone core and of regulatory proteins to DNA involving both the major and the minor grooves (Van Holde et al., 1974; Church et al., 1977; Carter & Kraut, 1974; Seeman et al., 1976; Adler et al., 1972; Richmond and Steitz, 1976). Up to now, there have been no direct experimental data supporting these models. The common feature of these models is that the histones are bound by their basic N-terminal residues to phosphates, while the recognition process in eucaryotes occurs through the interaction between the base atoms and the amino acid side chains. Data from protamine–DNA complexes are consistent with the model of protamine polypeptide chain bound to DNA along its minor groove (Feughelman et al., 1955; Wilkins, 1956; De Santis et al., 1974; Subirana & Puigjaner, 1973; Suau & Subirana, 1977; Herskovits & Brahms, 1976).

Since, at present, chromosomal nonhistone proteins are not yet individually characterized and separated, it is only possible to study them globally. We have recently suggested on the basis of chromatin investigation that the NHP interact within the large groove of DNA, while the histones of the nucleosome core do not (Goodwin & Brahms, 1978). In the present investigation, the structures of nucleosomes, and also of chromatins of different origins and different degrees of activity with regard to gene expression, were compared using Raman scattering. The studies of the site of attachment of the methyl group in DNA using dimethyl sulfate (Me_2SO_4) allowed one to assign some DNA binding sites of the proteins, NHP and histones.

Materials and Methods

Raman Spectra. Raman spectra were obtained on a Jarrell-Ash 25-400 Laser raman spectrophotometer equipped with a Coherent-Radiation Model CR-4 ionized argon laser using 400–500 mW of power from the 514.5-nm line.

In order to take Raman measurements, the sample was placed either in a quartz capillary (diameter 2.5 mm) or in a Debye-Scherrer X-ray diffraction tube. This was placed in a specially constructed brass block through which water of a controlled temperature was passed. The temperature of the brass block was maintained at 10 °C in all the experiments discussed here. Nucleosome samples are unlike chromatin samples which are optically clear. They yield well-resolved Raman spectra with a relatively small background without observable Rayleigh scattering and, for quantitative measurement (see Table II), the base-line corrections are simple to introduce.

Preparation of Nucleosomes. Nuclei from different cells were prepared from rat liver by the method of Chauveau et al. (1956), from Zajdela hepatoma cells by the method of Reeder (1973), modified by De Pomerai et al. (1974), from chicken erythrocytes by the method of Spohr et al. (1972), and from calf thymus by the method of Shaw et al. (1974). Digestion was carried out to about 7% acid solubility or 2.5% in the case of calf thymus, and the resulting nucleosomes were separated on a Bio-Gel A-5m, 200–400 mesh, column eluted with a solution containing 10 mM Tris, 0.7 mM EDTA, 0.2 mM $PhCH_2SO_2F$, adjusted to pH 7.3 with sodium cacodylate.

† From the Institut de Recherche en Biologie Moléculaire (CNRS), Faculté des Sciences Tour 43, 2 Place Jussieu, 75221 Cedex 05, Paris, France. *Received August 10, 1978; revised manuscript received November 30, 1978.*

‡ Institut de Pathologie Moléculaire, Groupe inserm 137, 24 rue du Faubourg St-Jacques, 75014 Paris, France.

[1] Abbreviations used: Me_2SO_4, dimethyl sulfate; $NaDodSO_4$, sodium deodecyl sulfate; NHP, nonhistone proteins; $PhCH_2SO_2F$, phenylmethanesulfonyl fluoride.

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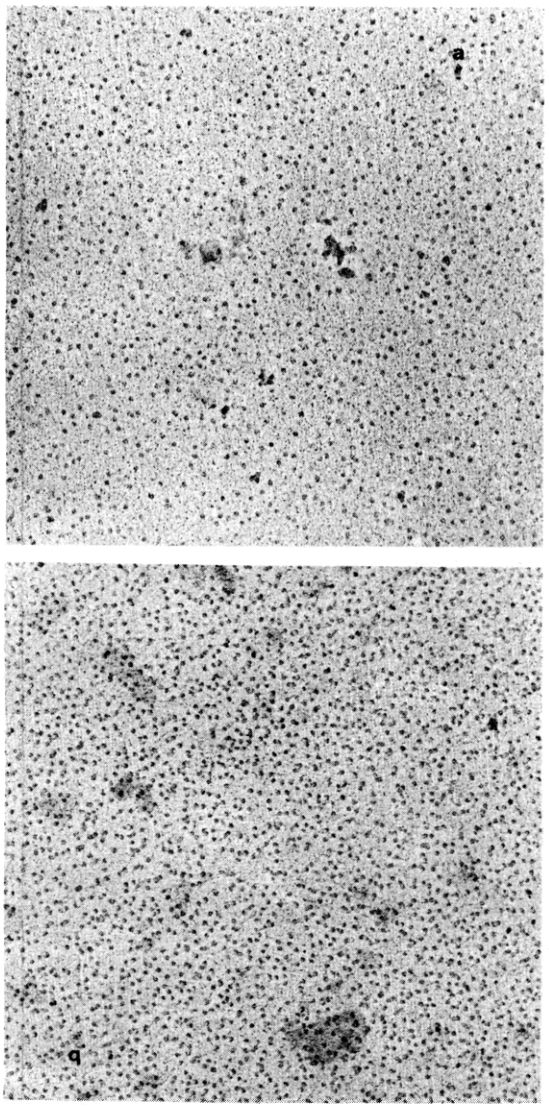

FIGURE 1: Electron micrographs of calf thymus nucleosome preparation. (a) Mononucleosome; (b) dinucleosome.

Several distinct peaks were present in the eluted material. Column fractions were combined to give different fractions containing essentially either mononucleosomes or mononucleosomes and dinucleosomes, or trinucleosomes and higher order particles. This latter fraction we referred to as polynucleosomes. Both of the above fractions are well separated from the excluded peak containing only digested chromatin.

The two fractions were concentrated by ultrafiltration in an Amicon cell and under vacuum in a dialysis bag to give a final concentration, in terms of DNA, of around 15–20 mg/mL. The nucleosomes were identified by electron microscopy (Figures 1a and 1b) and by gel electrophoresis.

Chromatin Preparation. Chromatin was prepared from different tissues according to the method of Reeder (1973), modified by De Pomerai et al. (1974), which appeared to be the most suitable method for obtaining high molecular weight native chromatin without shearing. Chromatin was suspended in 2 mM NaCl, 0.2 mM $PhCH_2SO_2F$, and 0.1 mM EDTA and dialyzed against the same solution overnight.

Gel Electrophoresis. Fifteen percent $NaDodSO_4$–polyacrylamide gels were prepared according to the method of Laemmli (1970). The nucleosomes were dissociated by $NaDodSO_4$. Electrophoreses were carried out at constant current of about 0.7 mA per gel. Gels were stained with Coomassie brilliant blue. Gel scanning was carried out on a spectrometer constructed in the laboratory for this purpose, which allowed the tracing of protein bands using 510-nm light as an excitation source. The results are expressed as a function of the migration of bromophenol blue used as a marker (R_f).

Methylation of Nucleic Acids. The methylation of calf thymus DNA (Worthington) and of poly(A) (Miles) was carried out with dimethyl sulfate (Me_2SO_4) in 0.2 M sodium cacodylate in D_2O at pD of about 8. Pure Me_2SO_4 was added to the nucleic acid solution in the amount of 1 mol per mol of nucleotide residue. After incubation for about 30 min at room temperature, each mixture was cooled to 0 °C and the methylation reaction proceeded for about 20 h for poly(A) and for longer period up to 120 h for DNA.

Deuterium Exchange. In order to facilitate the observation of certain bands which are obscured by the contribution of the water band and to improve the base-line correction, the samples were submitted to deuterium exchange. Deuterated nucleosomes were prepared by introducing a quantity of D_2O into the capillary tube containing the aqueous solution of nucleosomes which is then sealed. The D_2O is changed every 4–5 h and the exchange is allowed to proceed for periods of up to 60 h.

Results

Raman scattering data have been obtained from nucleosomes from a variety of tissues having different degrees of activity with respect to gene expression in order to obtain new information on the sites and types of interactions of DNA with proteins. It was found recently that nucleosomes from rat liver and other biologically active tissues contain not only core histones but also nonhistone proteins (Defer et al., 1978). Since chromatin samples are usually more or less turbid, nucleosomes are more suitable for Raman scattering studies. In addition, we used some modified DNA and polynucleotides as an aid to the study of the sites of DNA–protein interactions. In parallel with the Raman investigation, nucleosomes and chromatins were studied by electrophoresis on polyacrylamide gels in dissociating media which show in a quantitative manner the protein composition of the material used.

I. Nucleosomes from Rat Liver. Figure 2a shows the Raman spectrum obtained from mono- and dinucleosomes while Figure 2b is the spectrum of polynucleosomes, i.e., tetra and higher order nucleosomes. Corresponding polyacrylamide gel electrophoresis results are presented in Figures 3a and 3b. It is clear from the electrophoregrams presented that each fraction has a good complement of core histones and of H1 and has a detectable amount of nonhistone proteins (NHP). The amount of NHP present is not as great as that observed in total chromatin from rat liver but is clearly greater than that observed in the nucleosomes from chicken erythrocytes (see below).

The bands in the Raman spectrum of mononucleosomes are particularly well resolved. This spectrum is dominated by the DNA contribution but the amide III and I bands of the polypeptide backbone vibrations are also observed. The assignment of Raman bands based on previous studies of Peticolas et al. (1971), Small and Peticolas (1971), Lord & Thomas (1967), Tsuboi et al. (1973), and Goodwin & Brahms (1978) is listed in Table I. In the region of 1200–1600 cm^{-1}, one observes several bands characteristic of base vibrations, some of which have their intensities significantly reduced with respect to those observed from DNA. This will be considered in more detail below.

One can clearly distinguish the presence of a band at 835 cm^{-1} assigned to phosphodiester backbone vibrations whose frequency indicates that DNA is in a B-type conformation and

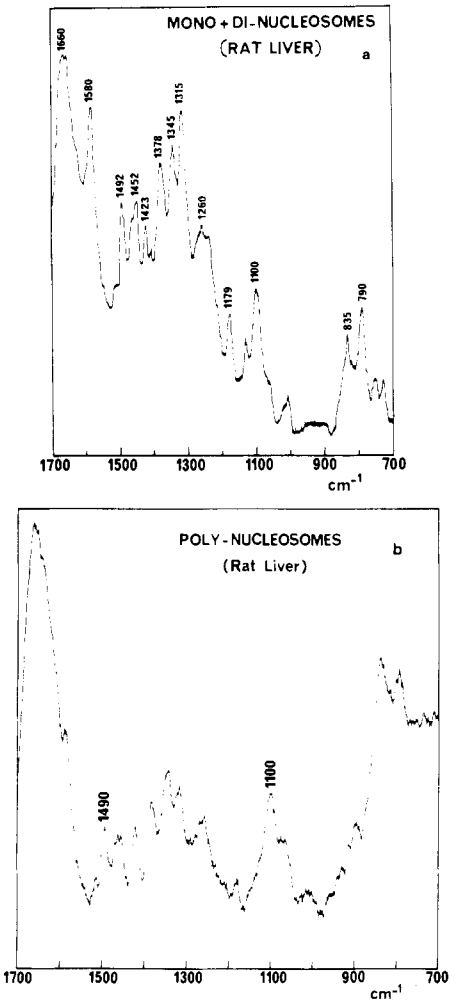

FIGURE 2: Raman spectra of nucleosomes from rat liver. In a is shown a spectrum obtained from a fraction containing essentially mononucleosomes, while spectrum b is of the polynucleosome fraction (see text) from the same preparation. Both samples contained approximately 20 mg/mL of DNA. Solvent: 10 mM Tris, 0.7 mM EDTA, 0.2 mM $PhCH_2SO_2F$, pH 7.3.

Table I: Identification of Raman Band Frequencies for Nucleosomes, Chromatin, and DNA

in H_2O	in D_2O	assignment
807 (form A)		
803–835 (form B)	830–835	DNA backbone
860–870 (form C)		O–P–O diester stretch
892	897	ribose phosphate
1015	1025	C–O stretch
1058		C–O stretch
1096	1096	O–P–O symmetrical stretch
≃1145	broad D_2O band at 1210 cm^{-1}	deoxyribose phosphate base C–N
≃1180	1260	stretch
1260	1306	C, A, amide III protein
1306	1350	A
1341	1381	A
1378	1429	T, A, G
1428		A, G
1455		C–H deformations (protein)
1490	1485	G, A
1514	1524	A
1580	1578	A, G
1650–1660		amide I (α helix)

that there is no other detectable form. At 1660 cm^{-1} one observes the presence of an amide I band which indicates that the proteins are predominantly in the α-helical conformation. These results are in general agreement with previous results

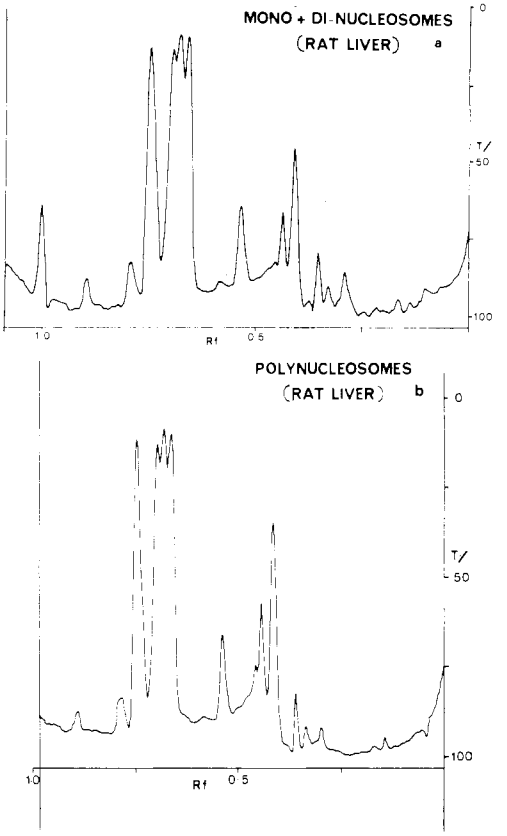

FIGURE 3: Polyacrylamide gels of rat liver nucleosomes. Profiles of the stained polyacrylamide gels obtained after electrophoresis of the rat liver nucleosome preparation whose Raman spectra are presented in Figure 2a from the mononucleosome fraction and in Figure 2b from the polynucleosomes.

Table II: Ratio of the Intensities of the Bands at 1490 and 1580 cm^{-1} with Respect to the 1100-cm^{-1} O–P–O Stretch Band

material	1490 cm^{-1}	1580 cm^{-1}
DNA calf thymus[a]	1.1	1.3[b]
polynucleosomes chicken erythrocytes	1.0	
mononucleosomes chicken erythrocytes	1.1	
polynucleosomes calf thymus	1.1	0.8[b]
mononucleosomes calf thymus	1.2	
polynucleosomes rat liver	0.6	
polynucleosomes rat liver		0.9
mononucleosomes rat liver	0.8	
mononucleosomes rat liver		0.9
polynucleosomes Zajdela hepatoma cells	1.1	
mononucleosomes Zajdela hepatoma cells	1.0	
chromatin calf thymus	0.6	
chromatin rat liver	0.4	
chromatin Zajdela hepatoma cells	0.2	
protamine-DNA (1/1, w/w)[d]	1.1	0.9[b]
clupeine-DNA (1/1, w/w)[d]	1.0	0.8[b]
DNA methylated	0.3	0.5
adenine	0.3	0.3[b,c]
3-methyladenine	0.3	0.2[b,c]

[a] Average value obtained from about five preparations. [b] Value obtained from D_2O solution. [c] Ratio obtained with respect to the cacodylate band at 610 cm^{-1}. [d] Spectra presented by Herskovits & Brahms (1976).

on chromatin and also nucleosomes from calf thymus (Goodwin & Brahms, 1978) and from chicken erythrocytes (Thomas et al., 1977).

It is interesting to note that in nucleosomes from rat liver there is a significant reduction in the intensity of the band at about 1490 cm^{-1} in comparison with that observed for free DNA (Table II). The intensity ratio of the 1490-cm^{-1} band,

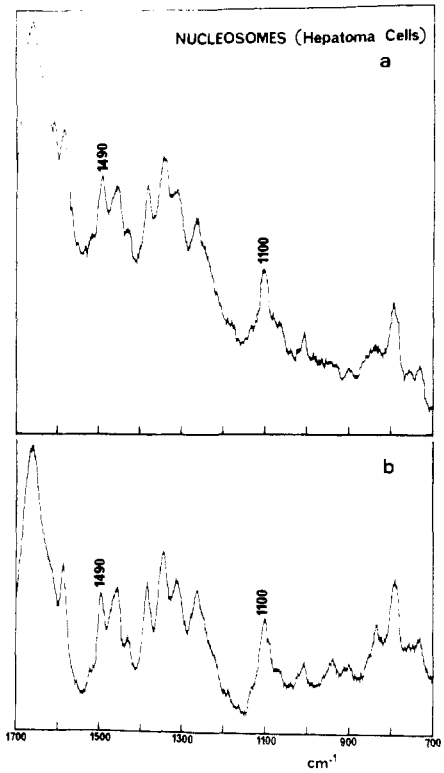

FIGURE 4: Raman spectra from nucleosomes of Zajdela hepatoma cells. The results from a mono- and dinucleosome fraction (a) and a polynucleosome fraction (b) are shown.

assigned essentially to guanine, to that at 1100 cm^{-1} due to the phosphate O=P=O symmetric stretching vibration taken as an internal standard, is shown in Table II. This value in DNA is slightly greater than 1 and is reduced in mono- and polynucleosomes from rat liver to about 0.6–0.8. The decrease in the intensity of the 1490 cm^{-1} band is still much more pronounced in chromatin from rat liver (Table II). This reduction in the intensity of the band at about 1490 cm^{-1} is correlated with the content of NHP and is proposed to reflect their interaction at the guanine N(7) position (see below and Goodwin & Brahms, 1978).

Calculation of the relative intensity of the band at 1378 cm^{-1}, using the phosphate O=P=O band at 1100 cm^{-1} as an internal standard, indicates a decrease of the intensity of about 40% with respect to DNA. However, this band is of mixed assignment being attributed to thymine, adenine, and guanine ring mode vibrations. It is difficult, therefore, at present to ascribe this change to a definite structural feature of the nucleosomes or chromatins. The relative decrease of intensity of this band may reflect some fundamental feature of the nucleosome structure since the reduction is quantitatively similar in all nucleosomes preparations.

Another band of particular interest in nucleosomes is the 1580-cm^{-1} band which in DNA is assigned predominantly to adenine with some contribution from guanine vibrations. The quantitative investigation of its intensity is difficult in aqueous solution due to the contribution of H_2O bands. The changes of the intensity of the 1580-cm^{-1} band will be considered in detail on deuterated nucleosomes.

II. Nucleosomes from Zajdela Hepatoma Cells. Figure 4 shows the Raman spectra obtained from mono- and polynucleosomes isolated from Zajdela hepatoma cells.

Preparations of whole unsheared chromatin from the same material contain a large NHP content and gave Raman spectra in which there is a marked reduction in the intensity of the band at 1490 cm^{-1} as compared with DNA (see Table II). The nucleosome preparations from hepatoma cells contain much less NHP, probably due to loss during preparation, and the reduction in the intensity of this band is relatively very small indeed. A similar small reduction is also observed in spectra from calf thymus and chicken erythrocyte nucleosomes, which have very low content of NHP. In contrast, a marked decrease in intensity was observed in the spectra from rat liver nucleosomes which have high NHP content.

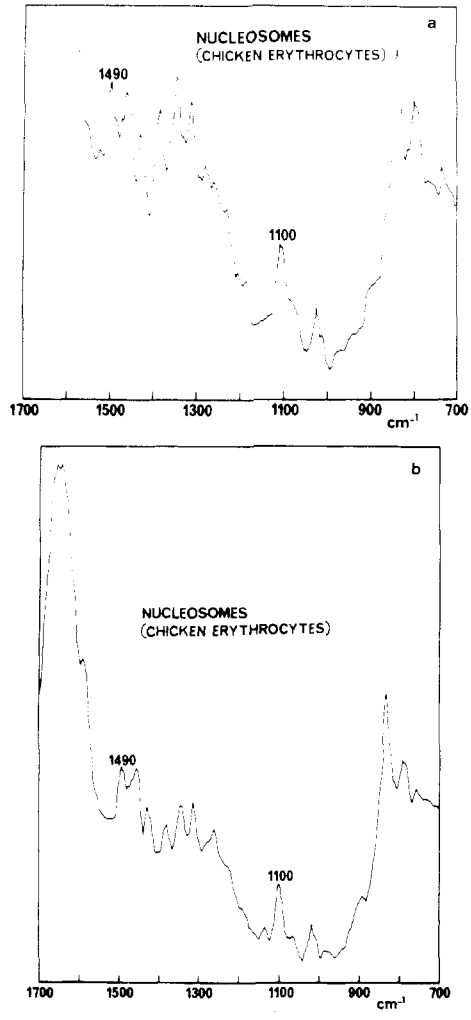

FIGURE 5: Raman spectra from chicken erythrocyte nucleosomes. (a) Mono- and dinucleosomes and (b) polynucleosomes.

III. Nucleosomes from Chicken Erythrocytes. A Raman spectrum obtained from chicken erythrocyte mononucleosomes is presented in Figure 5a, while in Figure 5b a corresponding spectrum obtained from polynucleosomes is shown. Polyacrylamide gel electrophoregrams of the same material are presented Figures 6a and 6b.

Erythrocytes are examples of cells having a low activity with regard to gene expression. It can be seen from the electrophoresis results that, although the material has a full complement of core histones, there are no detectable amounts of NHP. In addition, an important difference between the mononucleosome and polynucleosome preparations is that there is a large H5 component in the polynucleosomes but no detectable amount in the mononucleosome fraction. The ratio of the band at 1490 cm^{-1} to that at 1100 cm^{-1} is shown in Table II. The value of this ratio is near 1 in mononucleosomes and polynucleosomes and is not significantly different from that observed in DNA. The similarity of the value of this ratio from both fractions which have significantly different relative

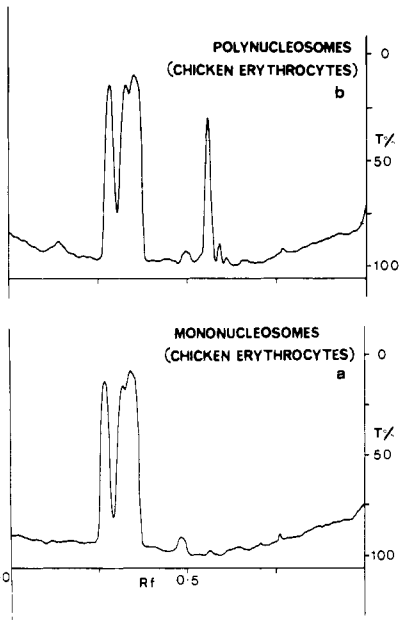

FIGURE 6: Electrophoretic profiles of nucleosomes from chicken erythrocytes. (a) Mononucleosomes and (b) polynucleosomes.

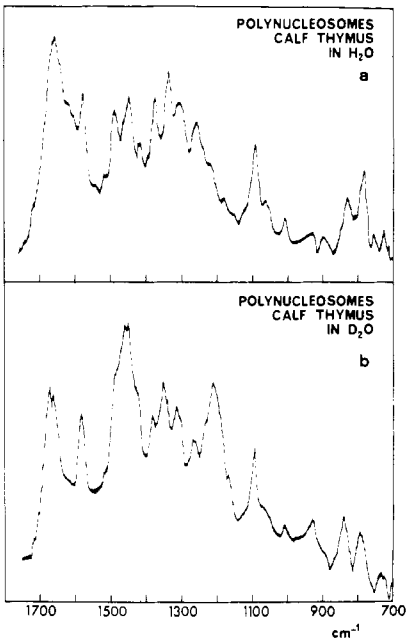

FIGURE 7: Raman spectra from calf thymus polynucleosomes. Curve a shows the spectrum obtained from the fraction directly after preparation in H_2O solution, while b shows a spectrum obtained from the same material after deuteration by exchange, as described in Materials and Methods. In addition to the D_2O band at about 1200 cm^{-1}, a broad intense band at around 1450 cm^{-1} is observed due to HDO.

amounts of H5 strongly suggests that this protein and also H1 are not responsible for intensity changes observed in the 1490-cm^{-1} band.

IV. Nucleosomes from Calf Thymus. Nucleosomes from calf thymus have been studied in D_2O solution. This allows one to observe Raman bands which in aqueous solution are totally or partially obscured by the broad H_2O band at around 1700 to 1500 cm^{-1}. This spectrum from deuterated nucleosomes is compared with one obtained in aqueous solution (Figure 7). In order to avoid structural changes resulting from freeze-drying, nucleosomes were prepared in aqueous solution and were subsequently submitted to exchange with D_2O as described in the Methods. Since this involves not only the exchange of the labile protons on the DNA and proteins but also of the water constituting the solvent, it was not possible to replace entirely the H_2O by D_2O and, hence, a strong HDO band at around 1450 cm^{-1} is observed as well as the D_2O band at around 1200 cm^{-1}. However, the band at about 1580 cm^{-1} resulting from adenine and guanine vibrations and which is normally partially obscured by the water band is now well resolved and its relative intensity may be measured.

The values obtained for the intensity of this band with respect to that at around 1100 cm^{-1} due to the phosphate symmetric stretching are presented in Table II as is the value observed for the same ratio in the spectra from calf thymus DNA. There is a reduction in this ratio in nucleosomes when compared with DNA of around 30–40%. This is a quantitatively similar effect to that observed in spectra from DNA–protamine and DNA–clupeine complexes (see Table II).

In the chicken erythrocyte nucleosomes discussed above, the differing amounts of H5 in the two fractions did not affect the intensity of the 1490-cm^{-1} band. In order to determine whether the intensities of either the 1490- or 1580-cm^{-1} band are affected, we have performed some preliminary experiments to test the effect of the direct addition of H1 to calf thymus nucleosomes. The spectra (not shown), obtained from both aqueous and deuterated samples, show no effect upon either the 1490-cm^{-1} band or the 1580-cm^{-1} band.

V. Identification of the Sites of Methylation (by Me_2SO_4) in Nucleic Acids. In order to obtain information on the possible sites of interaction of DNA with proteins, it was of interest to perform a Raman study of methylated nucleic acids. It was established by Lawley & Brookes (1963) (for a review, see Lawley (1966) and Singer (1975)) that the alkylation reaction of DNA carried out with dimethyl sulfate (Me_2SO_4) leads to methylation at the N(7) of guanine and the N(3) of adenine. The N(1) atom of adenine is available for methylation only in single-stranded DNA. Recently it was shown by Raman scattering studies that the methylation of guanine at the N(7) position either in DNA or in GMP leads to a pronounced decrease in the intensity of the 1490-cm^{-1} Raman band (Mansy & Peticolas, 1976; Goodwin & Brahms, 1978). It was of importance to identify the methylation site in adenine and correlate this with the spectral changes in Raman bands.

The methylation of DNA and poly(A) was carried out with dimethyl sulfate (pH 7.5), at 0 °C for up to 125 h (see Materials and Methods) following the general procedure of Lawley & Brookes (1963). The reaction was carried out in D_2O solution which permitted intensity measurements to be taken from the bands in the 1700–1550-cm^{-1} region (Figure 8).

The bands at 1490 cm^{-1} and at 1580 cm^{-1} were of reduced intensity (about 40% and 50%, respectively) in the methylated DNA and no significant changes in the intensities of other bands were observed. One may also observe that upon methylation the 1580-cm^{-1} band decreases strongly its intensity and shifts to higher frequency at about 1610 cm^{-1} which is shown in more detail by our current kinetic studies of DNA methylation (Goodwin & Brahms, in preparation). The sites of methylation of DNA under these conditions are known to be the N(3) position of adenine and the N(7) position of guanine (Lawley & Brookes, 1963; Maxam & Gibert, 1977). In DNA the bands at 1580 cm^{-1} and 1490 cm^{-1} come predominantly from adenine vibrations and from guanine vibrations, respectively (Table I; see also Mansy & Peticolas, 1976). Hence, it is likely that the intensity reduction of the 1580-cm^{-1} band in DNA is primarily due to the substitution

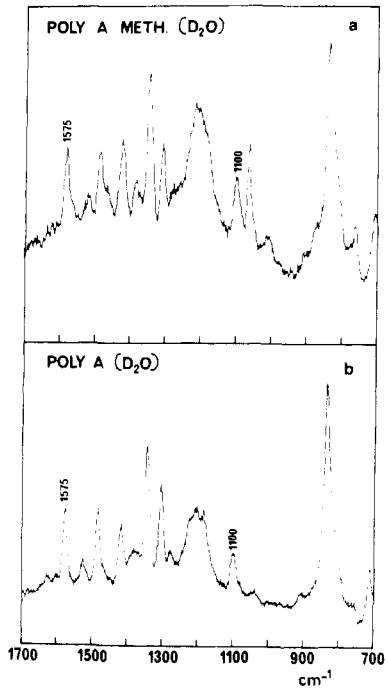

FIGURE 8: Raman spectrum from methylated calf thymus DNA. Raman results obtained from methylated calf thymus DNA with Me_2SO_4 after 24 h as described in Materials and Methods (curve b) and for comparison (curve a) the spectrum of the unmodified DNA control. The solvent in both cases was 0.2 M cacodylate in D_2O. Two intense bands due to the dimethyl sulfate are also visible in the spectrum from the methylated sample in addition to the D_2O band at about 1200 cm^{-1}.

at the adenine N(3) position. Since the 1490-cm^{-1} band in DNA arises primarily from guanine vibration, it also appears likely that the methylation of guanine at the N(7) position is responsible for the intensity reduction in this band.

Since the N(3) of adenine is the site of methylation in DNA by Me_2SO_4 (Lawley & Brookes, 1963), we investigated 3-methyladenine taken as a model. The results of the measurements of the intensity of the 1580-cm^{-1} Raman band in 3-Me-Ade and in AMP, relative to the 610-cm^{-1} band of cacodylate used as an external standard, indicate a reduction of about 30–40% (Table II). In order to test whether the substitution at N(1) is responsible for the observed changes in the 1580-cm^{-1} band, 1-Me-Ade was investigated; the intensity of this band is unchanged in this compound in comparison with AMP.

A previous investigation led to the conclusion that: "DNA fully alkylated with HN_2 nitrogen mustard both in H_2O and in D_2O shows no measurable changes in the Raman spectrum of the adenine bands" (Mansy & Peticolas, 1976). This is most probably due to differences in the methylation technique. In the present studies, a different reagent (Me_2SO_4) was used, the reaction was allowed to proceed for periods of up to 125 h and was performed in D_2O solution which gives better resolution of bands around 1600 cm^{-1}. Furthermore, previous studies (Lawley & Brookes, 1963; Maxam & Gilbert, 1977) have shown that the methylation of adenine is five times slower than that of guanine.

Two conclusions can be drawn. (1) The band at about 1580 cm^{-1} of DNA, in view of these results and previous work on methylation of Lawley & Brookes, may be assigned to adenine vibrations involving predominantly the N(3) position. (2) The band at 1490 cm^{-1} whose intensity is strongly decreased in 7-methylguanosine with respect to GMP (Goodwin & Brahms, 1978; Mansy & Peticolas, 1976) and in DNA methylation either with Me_2SO_4 (Figure 8) or with the difunctional methyl nitrogen mustard (Mansy & Peticolas, 1976) results mainly from N(7)=C(8) stretching vibrations. The methylation at N(7) or protonation at N(7) at pH 1 reduces its intensity or causes its disappearance (Lord & Thomas, 1967).

One may thus conclude, on the basis of these Raman results combined with the previous methylation data of Lawley & Brookes (1963), that the bands at 1490 and 1580 cm^{-1} of DNA are useful parameters for detection of molecular interactions at the N(7) position of guanine exposed to the large groove and at the N(3) position of adenine exposed to the groove (Figure 9).

Discussion

The data presented here have been obtained from the nucleosomes of different eucaryotic cells characterized by different degrees of cellular activity with respect to gene expression and having different amounts of chromosomal nonhistone proteins (NHP). Two main lines of direct evidence indicate that an attachment of histones to DNA bases takes place in the minor groove, and that, in the absence of NHP, the large groove is unoccupied, and that there is a site of interaction of the NHP on DNA bases in the large groove.

(1) The intensity of the Raman band at about 1490 cm^{-1} is strongly decreased with respect to DNA in nucleosomes and chromatins extracted from tissues which are relatively rich in NHP, e.g., rat liver. In contrast, in nucleosomes which have a very low NHP content, e.g., chicken erythrocytes and calf thymus, the intensity of this band is essentially similar to that of DNA. H1 is not responsible for the observed changes since its presence or addition has no influence on the intensity of this band. The 1490-cm^{-1} band is sensitive to substitution, methylation, or protonation at the N(7) position of guanine and therefore reflects DNA interactions at this position which in B DNA is exposed to the large groove (see Table II and Mansy & Peticolas, 1976; Goodwin & Brahms, 1978; Lord & Thomas, 1967). Since NHP are considered as including proteins involved in gene expression, one may thus consider the interaction with bases situated in the large groove as specific.

(2) The 1580-cm^{-1} Raman band results from purine base vibrations and in DNA has been assigned predominantly to adenine vibrations.

Our DNA methylation results suggest that the particular adenine vibrations involved are around the N(3) position which lies in the small groove of DNA.

A reduction in the intensity of the 1580-cm^{-1} band has been observed in nucleosomes from different tissues. In particular, the preparation of calf thymus nucleosomes used in the present study did not have a detectable NHP component and no significant reduction in the intensity of the 1490-cm^{-1} band was observed. A decrease in the intensity of the band at 1580 cm^{-1} was observed.

Supporting evidence for this conclusion comes from Raman results on protamine–DNA complexes. A reduction in the intensity of the 1580-cm^{-1} band is observed in the Raman spectra of these complexes (spectra in Herkovits & Brahms, 1976) and when compared with spectra from DNA, though there is no decrease in the intensity of the band at 1490 cm^{-1} (see Table II). There is a good evidence from other sources (Wilkins, 1956; Feughelman et al., 1955; Suau & Subirana, 1977) that protamine binds to DNA in the small groove.

Raman spectroscopy appears to be a direct method which allows one to detect interaction between protein with a base situated either in the major or in the minor groove of DNA in its native state. In contrast, the use of chemical probes may

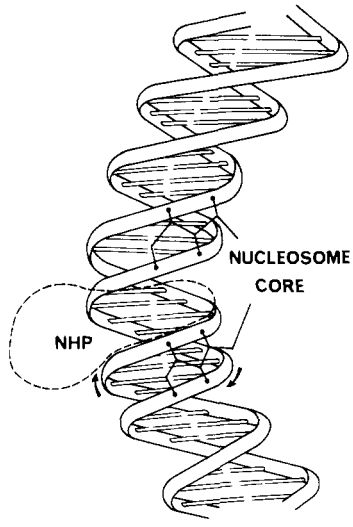

FIGURE 9: A schematic drawing of a possible partial view of a nucleosome. The interactions with DNA of two kinds of proteins, NHP and core histones, are shown.

either introduce modification or displacement of native constituents or may not yield information in the case of weak binding. Furthermore, due to the very complexity of chromatin structure, changes in the accessibility of the DNA to a chemical probe may be due to many factors.

Our results are consistent with a proposal that in nucleosomes and in chromatin there is a direct interaction between NHP and the base atoms exposed to the large groove, whereas an interaction between histones and DNA bases atoms occurs in the small groove (Figure 9).

The different relative intensities of the 1490-cm^{-1} band in chromatins from different origins could explain the apparent discrepancy between the two previous Raman studies of chromatins. Mansy et al. (1976) observed a reduction in the intensity of the 1490-cm^{-1} band in Raman spectra of mouse myeloma chromatin, whereas Thomas et al. (1977) observed no change in this band in Raman spectra from chicken erythrocyte chromatin with respect to DNA or after dissociation in 2 M NaCl solution.

Felsenfeld et al., using an entirely independent approach, found that in nucleosomes from chicken erythrocytes (containing no significant quantity of NHP) the large groove is unoccupied, which fully confirms our present Raman scattering results (Felsenfeld et al., 1978).

In conclusion, present comparative studies on nucleosomes and on chromatins from different origins allow one to observe, using Raman scattering, direct interactions between the bases in the minor and major grooves and the histones and NHP, respectively.

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Selective Inhibition of the Polymerase Activity of DNA Polymerase I. Further evidence for Separate Active Sites for Polymerase and 3′ to 5′ Exonuclease Activities†

Benito G. Que, Kathleen M. Downey,‡ and Antero G. So*

ABSTRACT: The chelating agent 1,10-phenanthroline, in the presence of a reducing agent, selectively inhibits the polymerase activity, but not the 3′ to 5′ exonuclease activity, of DNA polymerase I. The inhibition of DNA synthesis by 1,10-phenanthroline is potentiated by the addition of copper salts, as was previously reported by D'Aurora et al. [D'Aurora, V., Stern, A. M., & Sigman, D. S. (1977) *Biochem. Biophys. Res. Commun. 78*, 170]. Kinetic analysis demonstrates that a 1,10-phenanthroline–metal complex is a noncompetitive inhibitor of polymerase activity with deoxynucleoside triphosphate as the variable substrate and a competitive inhibitor with primer/template as the variable substrate. These results suggest that a 1,10-phenanthroline–metal complex competes with the primer terminus for the active site of the polymerase activity, but not the exonuclease activity, of DNA polymerase I. They also support our hypothesis that the polymerase and 3′ to 5′ exonuclease activities are catalyzed by separate active sites and that the primer terminus of the primer/template, which serves as a substrate for both activities, can bind to either of the catalytic sites. Some important implications follow from the delineation of separate active sites for the polymerase and 3′ to 5′ exonuclease activities with respect to the mechanism of DNA synthesis and the role of the 3′ to 5′ exonuclease activity in maintaining the fidelity of DNA synthesis.

Escherichia coli DNA polymerase I is a single polypeptide chain of 109 000 daltons which contains 1 g-atom of zinc/mol of enzyme (Springgate et al., 1973). The protein catalyzes several reactions including (1) extension of DNA chains in the 5′ to 3′ direction, (2) exonucleolytic degradation of DNA in the 5′ to 3′ direction, and (3) exonucleolytic degradation of DNA in the 3′ to 5′ direction. It is possible to separate the 5′ to 3′ exonuclease activity from the other activities of the enzyme by limited proteolysis (Brutlag et al., 1969; Klenow & Henningsen, 1970; Jacobsen et al., 1974). This treatment results in the splitting of the polypeptide chain into two fragments: a large fragment of 75 000 daltons which retains the polymerase and 3′ to 5′ exonuclease activities and a smaller fragment of 35 000 daltons which retains only the 5′ to 3′ exonuclease activity.

We have recently demonstrated that the catalytic sites for the polymerase activity and the 3′ to 5′ exonuclease activity of DNA polymerase I can be functionally dissociated by specific inhibitors of exonuclease activity (Que et al., 1978). We have shown that the 3′ to 5′ exonuclease activity can be selectively inhibited by nucleoside 5′-monophosphates, whereas the polymerase activity is not inhibited. In this report, we will present data showing that 1,10-phenanthroline selectively inhibits the polymerase activity of DNA polymerase I while the 3′ to 5′ exonuclease activity is not inhibited.

The chelating agent 1,10-phenanthroline inhibits a variety of DNA and RNA polymerases, many of which have been shown to contain stoichiometric amounts of zinc (Slater et al., 1971; Scrutton et al., 1971; Springgate et al., 1973; Valenzuela et al., 1973; Auld et al., 1975; Lattke & Wesser, 1977; Wandzulak & Benson, 1978). Dialysis of DNA polymerase I against excess 1,10-phenanthroline results in the gradual removal of zinc from the protein, accompanied by a corresponding loss of DNA polymerase activity (Springgate et al., 1973). However, the addition of 1,10-phenanthroline to a DNA polymerase assay results in instantaneous inhibition of polymerase activity, suggesting that inhibition of DNA polymerase activity can result either from the removal of zinc from the protein or from the formation of an enzyme–phenanthroline complex.

Inhibition of polymerase activity by 1,10-phenanthroline has been found to be relieved at high DNA concentrations (Slater et al., 1971), and it has been proposed that the enzyme-bound zinc interacts with DNA. It has further been proposed that zinc has a mechanistic role in nucleotidyl transfer reactions, coordinating and promoting the nucleophilicity of the 3′-hydroxyl group of deoxyribose of the primer terminus, thus facilitating the attack of the 3′-hydroxyl group at the α-

† From the Howard Hughes Medical Institute Laboratory, Departments of Medicine and Biochemistry, and the Center for Blood Diseases, University of Miami School of Medicine, Miami, Florida 33101. *Received September 14, 1978; revised manuscript received February 5, 1979.* This research was supported by grants from the National Institutes of Health (AM 09001 and GM 25394) and the National Science Foundation (PCM 77-17651) and in part by research funds given in memory of Mary Beth Weiss and Elizabeth Wenig.
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