THE CONFORMATION OF DNA AND PROTEIN WITHIN CHROMATIN SUBUNITS

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1. Introduction

The chromatin core particle consists of 140 base pairs of DNA wrapped around [1,2] an octameric histone core [3]. It has long been known from circular dichroism studies [4–7] that the DNA conformation is in some way altered by interaction with the histone protein. In order to explain the observed compaction ratio of the length of DNA associated with the subunit [8,9], it has been suggested that the DNA adopts a B-form structure with kinks at intervals of 10 base pairs [10,11], rather than assuming a configuration in which the double helix is continuously bent. The conformation of the histones within the chromatin subunit has not been studied; the protein contribution to the CD spectrum of the core particle cannot be assessed because the ellipticity of the DNA is poorly understood above 270 nm and may not be assumed at shorter wavelengths. In free solution, however, the complex of histones H2A, H2B, H3 and H4 ('core protein') is thought to be largely globular with mobile random coil regions at the NH_2-termini [12–14], although this has recently been questioned [15].

In this study we use ^{31}P NMR spectroscopy to compare the environment of DNA phosphate groups in the 140 base-pair core particle with that of the phosphates in the extracted DNA, and infrared spectroscopy to compare the secondary structure of core protein in situ with that of the complex isolated in 2 M NaCl [3,16]. We conclude that the strain at the phosphate groups of DNA in the core particle is unaltered from that in B-form DNA, and that the α-helical and random coil contributions to the isolated core-protein secondary structure are maintained within the chromatin subunit.

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2. Experimental

2.1. *Preparation of materials*

Chromatin core particles were prepared by digestion of chromatin released from chick erythrocyte nuclei (isolated according to Shaw et al. [17]) by gentle spinning in 0.1 M sucrose, 10 mM Tris–HCl, pH 7.5. Micrococcal nuclease (130 units/ml) digestion of chromatin (6–10 mg/ml) in the presence of 0.7 mM $CaCl_2$ was for 10 min at 37°C. The digest was fractionated on a zonal sucrose gradient [18].

The core-protein histone complex from monomer particles was prepared as described previously [12,19]. A typical preparation yielded protein at a concentration of 2–3 mg/ml. DNA was extracted from monomer particles by pronase–phenol treatment [20].

2.2. *Concentration of materials*

Appreciable concentrations of materials are necessary for NMR and infrared spectroscopy (1–4%); these were achieved for DNA by lyophilisation from water, for core protein by brief ultrafiltration (Amicon UM20 membrane), while core particles were pelleted into a 40% w/w sucrose cushion in a SW41

rotor. Extinction coefficients used to determine concentrations of materials were: DNA, $E^{1\%}_{1\,cm}$ 200 at 258 nm; core protein, $E^{1\%}_{1\,cm}$ 3.69 at 275.5 nm [21,22], and core particles, $E^{1\%}_{1\,cm}$ 93.12 at 258 nm [23].

2.3. ^{31}P NMR

Spectra were recorded at 36.4 MHz using a Bruker WH-90 Fourier transform spectrometer. A 15 μs pulse length was selected with a sweep width of 6kHz; the resulting free induction decays were collected in 4096 points and exponentially modified equivalent to an artificial spectral line broadening of 1.5 Hz. Fourier transformation was carried out in 8192 points to reduce digitisation errors. Shifts were measured from the resonance of an external trimethyl phosphite standard.

2.4. *Deuteration of samples for infrared spectroscopy*

Deuterium oxide (D_2O) from Fluorochem Ltd., England, was redistilled to remove metal ions. Salts used for solutions in D_2O were dried at 60°C, and the apparent p*D* of buffers was adjusted to 0.4 units less than the required pH [24]. DNA was deuterated by lyophilising three times from D_2O. Core protein was rotary dialysed against 5 × 100 ml 2 M NaCl, 10 mM CHES, p*D* 8.6 in D_2O, while core particles were dialysed at 4°C against 10 mM Tris–DCl, 0.7 mM EDTA, p*D* 7.1 for two weeks. Solutions were estimated to be at least 97% free of water on the basis of the intensity of the HDO absorption band near 3400 cm^{-1}.

2.5. *Infrared spectroscopy*

Spectra were run in a double beam Grubb-Parsons grating instrument in matched demountable cells having calcium fluoride windows and path length 30 μm, determined from interference fringes. Spectra were recorded over the frequency interval 1550–1750 cm^{-1} with a coarse-slit programme and slowest scan speed. The sample temperature was not raised above 28°C whilst in the beam.

2.6. *Thermal melting*

Thermal melting of core particles and their isolated DNA was carried out in 10 mM cacodylate, 0.7 mM EDTA, pH 7.5 at 258 nm in sealed cuvettes using a heating rate of 0.5°C/min. Absorbances were corrected for thermal expansion.

3. Results and discussion

3.1. *Purity of materials*

DNA extracted from monomer particles had a 260 nm : 280 nm ratio of 1.9, ellipticity of 9400 deg.cm^2.mol^{-1} and hypochromicity of 26%. Figure 1a shows the migration of this DNA on a 15%

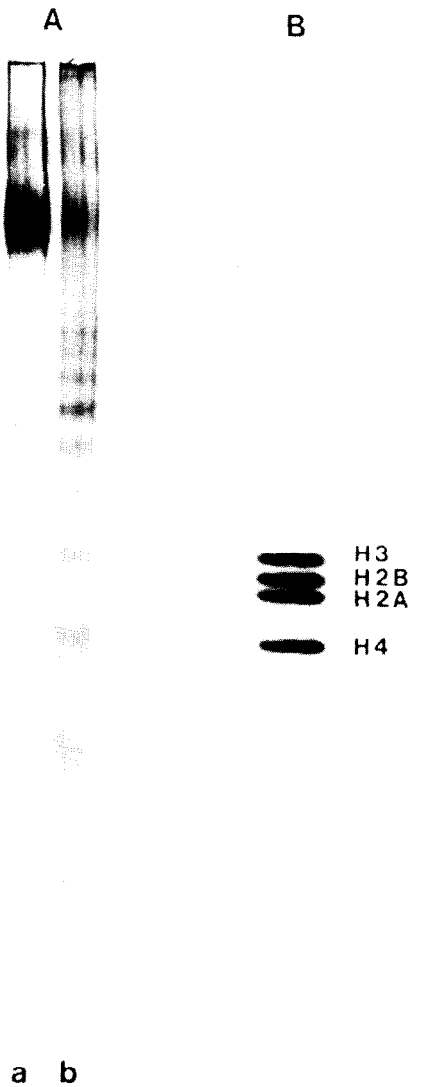

Fig.1. Purity of DNA and histones isolated from chromatin core particles: (A) 15% denaturing polyacrylamide slab-gel [25] showing: (a) DNA extracted from core particle (b) DNAase I digest of chromatin [26]. (B) 15% SDS–polyacrylamide gel [27] of salt-extracted histones from core particles [3,16].

denaturing polyacrylamide gel [25] alongside DNA fragments from a DNAase I digest of chromatin [26]. The absence of low molecular weight DNA demonstrates the integrity of both DNA strands in the core particle. Core particles were screened before and after the NMR experiments and lengthy deuteration procedures, and found to sediment at 11 S, melt with a T_m of 82°C in 10 mM cacodylate, 0.7 mM EDTA, pH 7.5 and to have a hypochromicity of 25% and ellipticity of 1850 deg.cm^2.mol^{-1} at 284 nm. The extracted core protein used in this study is shown to be lacking in histones H1 and H5 when run on a 15% SDS polyacrylamide gel [27] (fig.1b), and has less than 0.1% DNA contamination as judged by diphenylamine reaction [28] and lack of disymmetry of scattered laser light [19]. The core protein sediments at 3.8 S ± 0.1 S.

3.2. ^{31}P NMR

The ^{31}P NMR spectra of core particles and their extracted DNA are shown in fig.2. The similarities in shift and linewidth (table 1) are striking.

The similarity between the linewidths of core particles and their purified DNA is not surprising since, although the molecular weight of the former is more than double the latter, the more globular shape of the core particle will result in increased isotropy of rotation. Considerably broader ^{31}P NMR spectra have been recorded from intact chromatin [29] where motional possibilities are reduced. The linewidth for DNA compares well with that observed for sonicated DNA [29]. The similarity in chemical shift for core particles and their extracted DNA is interesting since phosphate and phosphodiester ^{31}P NMR shifts are highly sensitive to distortion of tetrahedral geometry [30–35]. Both empirical [30,32] and theoretical [31] observations of Gorenstein

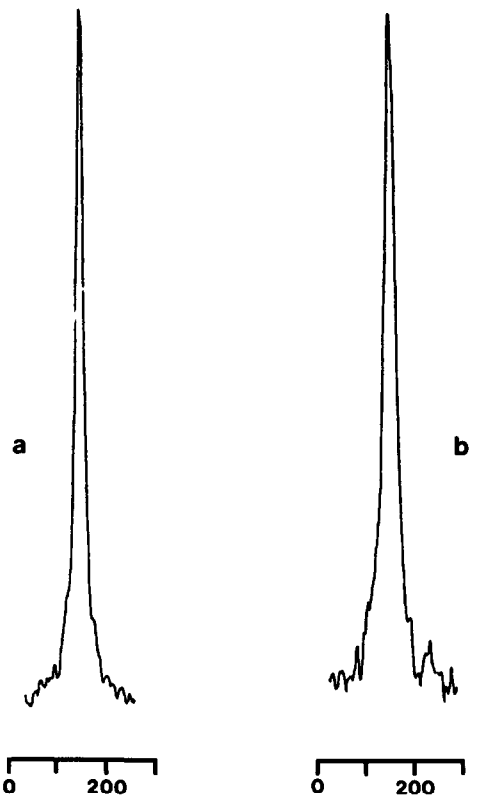

Fig.2. ^{31}P NMR spectra of (a) 140 bp DNA at 32°C and (b) 140 bp core particles at 42°C. Samples were studied in 10 mM Tris, 0.7 mM EDTA pH 7.5 at DNA concentrations of 10 mg/ml.

indicate a very steep dependence of ^{31}P NMR shifts upon alteration of O–P–O bond angle and bond torsional angle, i.e., effectively 'strain'. Changes in the O–P–O bond angles of phosphodiester compounds of two degrees can result in shifts of 10 ppm. The

Table 1
Comparison of spectral parameters of 140 base pair DNA and core particles

Sample	Temperature	Linewidth[a] (Hz)	Shift (Hz) (rel. to TMP)
DNA	32°C	21 ± 3	121
Core particles	42°C	27 ± 3	121

[a]Uncorrected for 1.5 Hz line broadening due to exponential modification

closeness of the shifts for DNA and core particles is to within one computer memory location, i.e., less than 0.1 ppm. A great 'spread' of resonances due to a variety of environments is also unlikely in view of the observed linewidths. Therefore the geometry of the phosphate groups of DNA in the core particles must be very similar to that of B-form DNA in solution.

We conclude that the O–P–O bond angles change by less than one degree on adoption of nucleosomal conformation.

3.3. *Thermal melting*

The derivative melting profiles of 140 base pair DNA in the core particle and in free solution are shown in fig.3. The thermal denaturation of histones will not contribute at this wavelength. Allowing for the well-known increase in T_m of DNA when interacting with histones, the derivative melts can be superimposed. The shoulder at 72°C shown by DNA melting within the core particle probably represents the melting of an *o, h* helix [36]. The sharp co-operative character and comparable hypochromicities of both melts makes an extensive contribution from single-stranded stacked regions unlikely in the DNA within the core particle.

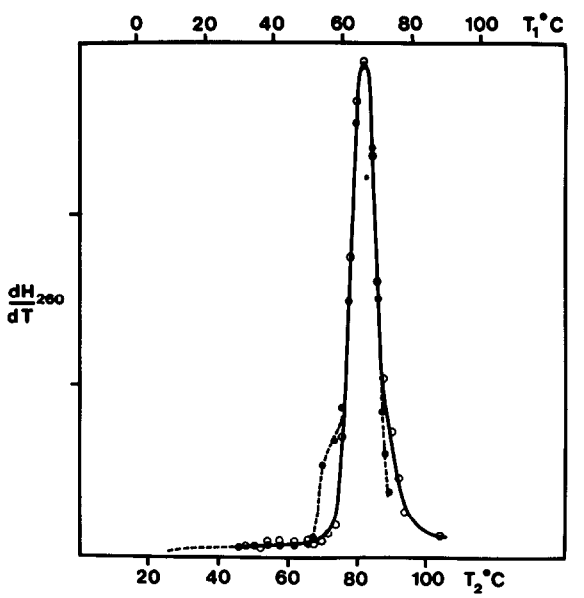

Fig.3. Derivative thermal melts of core particles and extracted DNA superimposed with respect to T_m: (- - -) Core particle, temperature scale T_2. (——) DNA from core particle, temperature scale T_1.

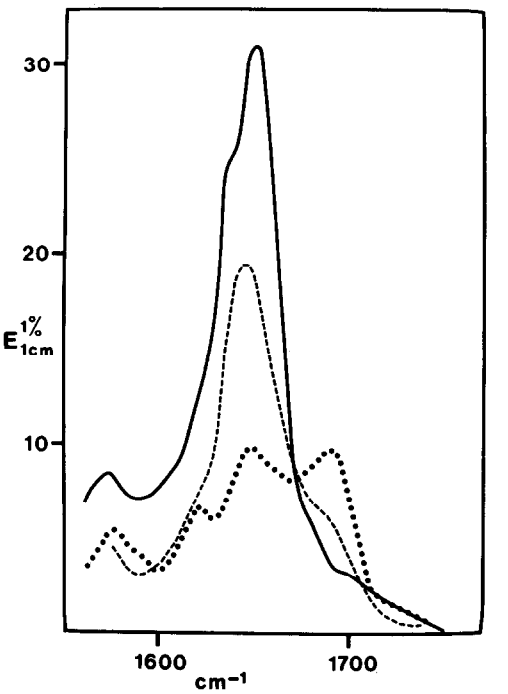

Fig.4. Infrared spectra from core particles and their constituents: (· · · ·) DNA in 10 mM cacodylate, 0.7 mM EDTA, p²H appt 7.1. (———) Core protein in 2 M NaCl, 10 mM CHES, p²H appt 8.6. (- - - -) Core particles in 10 mM cacodylate, 0.7 mM EDTA, p²H appt 7.1.

3.4. *IR Spectroscopy*

The infrared spectra from core particles and their constituents are shown in fig.4. Extracted DNA has the characteristic absorption in the region 1640–1700 cm^{-1} of in-plane stretching vibrations of the base residues [37] and is representative of a fully base-paired nucleic acid. The sharp amide I (C = O stretch) band at 1650 cm^{-1} of the core protein has an intensity and position associated typically with many native proteins with considerable α-helical contribution to their structure [38–40], and the observed shoulder at 1637 cm^{-1} is consistent with some random coil character [12–14].

In order to determine whether the spectrum of the core particle represents the sum of the extracted components, we assume from the melting results that there is no change in base-pairing of the DNA in situ. The appropriate proportion of the DNA spectrum ($E_{1\,cm}^{0.444\%}$) [20] is therefore subtracted

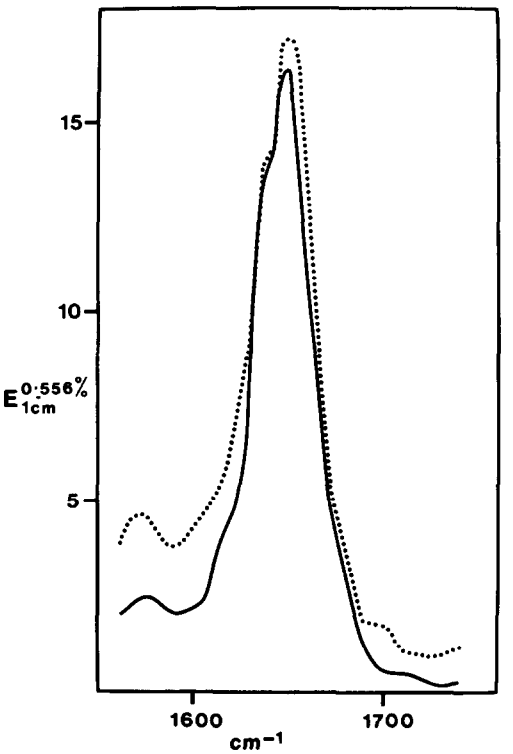

Fig.5. Infrared spectrum of core protein: (· · · ·) Experimental spectrum. (————) Spectrum calculated by subtraction of DNA spectrum from core particle spectrum.

from the core particle ($E^{1\%}_{1\,cm}$) spectrum. The resulting calculated ($E^{0.556\%}_{1\,cm}$) spectrum of core protein is shown in fig.5 alongside the experimental spectrum. The fit is seen to be good, except in the region below 1600 cm^{-1}, which approaches the bending mode frequency of the HDO species. Some HDO contamination from atmospheric exchange is hard to avoid and may influence the fit at these frequencies. It is, however, reasonable to conclude that the conformation of core protein is similar in situ and in the extracted state.

4. Conclusions

1. The environment and strain at the phosphate groups of DNA in the chromatin subunit are very similar to those of B-form DNA, as judged from their ^{31}P NMR spectra.
2. The extent of the core protein secondary structure is not affected by the removal of the DNA surrounding it. Random coil and α-helical contributions are maintained within the monomer core particle.

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