HISTONE INTERACTIONS WITH PROKARYOTE DNA

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1. Introduction

In eukaryotes, histones are found in association with DNA and are thought to act as non-specific gene repressors and to play an important role in controlling and maintaining the conformation of the chromosome [1]. Evidence for a superstructure in chromatin comes from X-ray diffraction patterns of fibres of nucleohistones which show a series of low angle rings [2, 3]. Similar low angle diffraction patterns have been observed for gels of nucleohistone [4]. An important observation in this work was the strong dependence of the intensities of the diffraction arcs on the concentrations of the gels. More recently it has been shown [5] that the very lysine rich histones can be selectively removed from both calf thymus and chicken erythrocyte chromatins without loss of any of the features of the low angle diffraction pattern; it was also found that the intensity of the 2.7 nm ring was very variable. These low angle patterns, which are not observed in the patterns of isolated DNA or total histone, have been attributed to the presence of a tertiary structure in the DNA–histone complex; possibly a supercoiled DNA structure [3, 6, 7]. Such higher order structure results entirely from histone interactions with DNA since the structure is found in reassembled complexes of DNA and isolated total histone. The major interaction appears to be between histones and the DNA phosphate-ester chain since Garrett [8] has reported the presence of very weak low angle diffraction rings in patterns from complexes of calf thymus total histone with prokaryote T7 DNA. No experimental evidence however was presented and it is important to confirm this observation and to demonstrate that prokaryote DNA–histone complexes can be formed which give X-ray diffraction patterns comparable with those obtained from eukaryote chromatin.

In other studies of prokaryote DNA–histone complexes, not involving X-ray diffraction, Rubin and Moudrianakis [9] have shown that calf thymus histone–coliphage λ_c DNA complexes have thermal stability, viscosity and fibre characteristics similar to those of native nucleohistone for histone/DNA w/v ratios greater than about unity, and that the histones bind co-operatively to the DNA. Olins and Olins [10] have studied KAP (F1) and GRK (F2A1) histones complexed with coliphage T7 DNA.

It is the purpose of this communication to demonstrate that X-ray diffraction patterns as strong and as well-defined as found for calf thymus nucleohistones can be obtained from complexes of prokaryote DNA with histones.

2. Methods and materials

Coliphage T7 DNA was kindly supplied by Dr. K. Cammack of the Microbiology Research Establishment, Porton Down, Salisbury, and coliphage $\lambda/\phi 80$ DNA by Dr. K. Murray of the University of Edinburgh. The T7 DNA had a hyperchromicity of 33% and a melting temperature of 74°C in 0.1 standard saline citrate buffer (SSC), in agreement with predictions using the formula of Owen et al. [11] for a G–C content of 48%.

Calf thymus nucleohistone was prepared using the method of Zubay and Doty [12]. From this preparation total histone was extracted using 0.25 M HCl with acetone precipitation and KAP (F1)-depleted histone was prepared by the method of Bradbury et al. [5]. Both histone preparations were characterized by polyacrylamide gel electrophoresis.

Two methods were used for producing the heterologous histone/DNA complexes. In both cases the histone was dissolved in distilled water; NaCl and urea were then added to give a final concn. of 2 M NaCl and 5 M urea. This solution was added drop-wise with constant stirring on ice [9, 13] to a solution of the coliphage DNA in 2 M NaCl and 5 M urea to give a histone/DNA w/w ratio 2/1.

In method 1 the final DNA concentration of the mixture was 0.7 mg/ml which was dialysed through the following steps: 1 M NaCl, 2.5 M urea (12 hr); 0.8 M NaCl, 2.0 M urea (6 hr); 0.6 M NaCl, 1.5 M urea (6 hr); 0.4 M NaCl, 1 M urea (12 hr); 0.2 M NaCl, 0 M urea (12 hr); 1 mM Tris buffer, pH 7 (12 hr).

In method 2 (following Olins and Olins [10], Bekhar et al. [14] and Slayter et al. [15] the final DNA concentration with histone in 2 M NaCl and 5 M urea was 1 mg/ml and the dialysis stages were: 1 M NaCl, 5 M urea (6 hr); 0.8 M NaCl, 5 M urea (3 hr); 0.6 M NaCl, 5 M urea (3 hr); 0.4 M NaCl, 5 M urea (12 hr); 0.3 M NaCl, 5 M urea (3 hr); 0.2 M NaCl, 5 M urea (3 hr); 1 mM Tris buffer, pH 7 (24 hr with many changes).

Samples for ultraviolet absorption spectrophotometry and thermal denaturation experiments and for electron microscopy were prepared from the solutions obtained by method 1 or method 2 after dialysis into 0.01 SSC buffer.

For electron microscopy drops of the diluted solutions at a DNA concn. 50 μg/ml were placed on carbon films on electron microscope grids and left for 30 min. The grids were fixed in 2.5% glutaraldehyde pH 7.4 stained with uranyl acetate and phosphotungstic acid and dehydrated in ethanol with final drying using 2-methylbutane or critical-point drying with CO_2.

Samples for X-ray diffraction and infra-red absorption spectroscopy were obtained by centrifugation in a 10 × 10 Ti rotor at 40 000 rpm for 2 hr. Fibres were prepared by pulling the gels with tweezers and they were studied at 94% humidity using a Searle X-ray camera with toroidal optics and quadrant apertures and stops at 98% relative humidity using a Frank low angle camera. The X-ray source used was a Hilger and Watts Y33 microfocus X-ray generator operated at 50 kV and 2 mA.

Infra-red absorption spectra were obtained from samples cast on polished barium fluoride plates.

3. Results

An X-ray diffraction pattern from a total histone–T7 DNA complex prepared by method 2 is shown in fig. 1a and a diffraction pattern from a complex of T7 DNA with all the histones except the KAP (F1) histone prepared by method 2 is shown in fig. 1b. A densitometer trace through the meridian of fig. 1a is shown in fig. 2. These two angle diffraction patterns are very similar, anf very clear diffraction rings can be observed centred at equivalent Bragg spacings 5.5, 3.6, 2.7 and 2.0 nm which correspond closely to those ob-

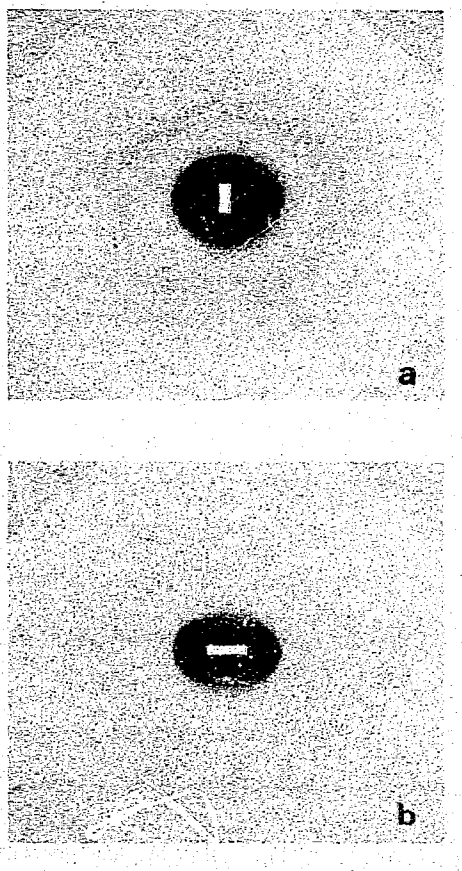

Fig. 1. a) X-ray diffraction pattern from total calf thymus histone complexed with coliphage T7 DNA by method 2. b) X-ray diffraction pattern from KAP (F1)-depleted calf thymus histone complexed with coliphage T7 DNA by method 1. In a and b the black patch near the back stop is scattering near 10 nm equivalent Bragg spacing and the first clear ring is at 5.5 nm.

served for native nucleohistone [2–5]. The intensity of the 2.7 nm ring is lower than that shown by Pardon and Wilkins [3]. We have found, however, in a wide range of nucleoprotein samples that the intensity of this ring is very variable; the factors involved in this variability are not fully understood. The low angle pattern of the complex of T7 DNA with total histone has also been recorded at 98% r.h. with the Frank camera. In this pattern in addition to rings at 5.4, 3.5 and 2.7 nm a ring is present at approx. 11.0 nm. This clearly accords to the diffraction arc at 11.0 nm observed by Luzzati and Nicolaieff [4] and Pardon and Wilkins [3] for gels.

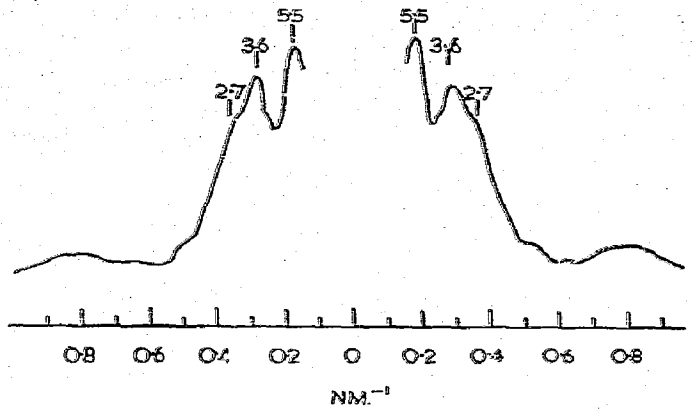

Fig. 2. A densitometer trace along the meridian of the diffraction pattern shown in fig. 1a. The quoted numbers are equivalent Bragg spacings (nm) for diffraction rings observed in the photographs.

An infra-red absorption spectrum of the total histone–T7 DNA complex mentioned above is shown in fig. 3 and another spectrum from native calf thymus nucleohistone is also shown in the figure for comparison. The amount of protein in a nucleohistone sample can be calculated by comparing the intensities of the two bands at 1542 cm^{-1} and 1240 cm^{-1} which have been assigned to the amide II band from proteins and the antisymmetric PO_2^- stretching vibration of DNA, respectively [16]. The native nucleohistone contains 56% protein and the total histone–T7 DNA complex contains about 45% protein.

Electron micrographs of the histone–prokaryote DNA complexes when prepared from buffered solutions

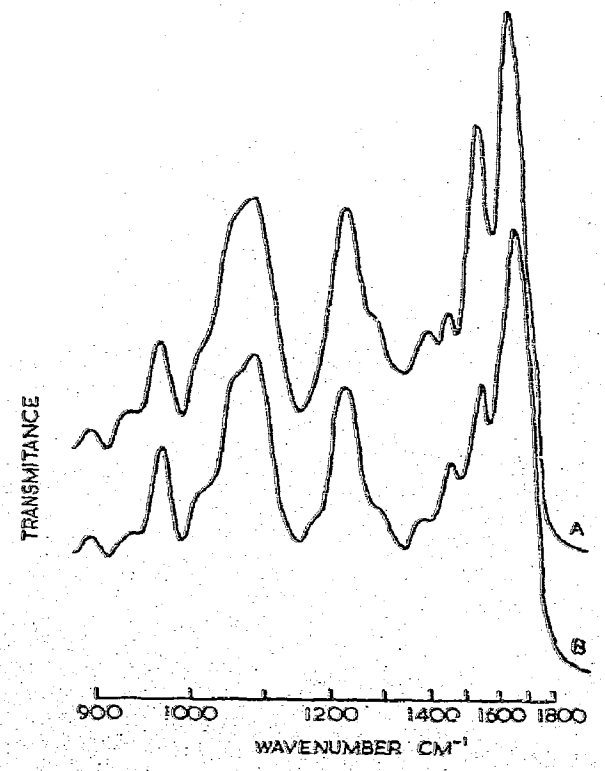

Fig. 3. Infra-red spectra of A) native calf thymus nucleohistones and B) calf thymus total histone–coliphage T7 DNA complex.

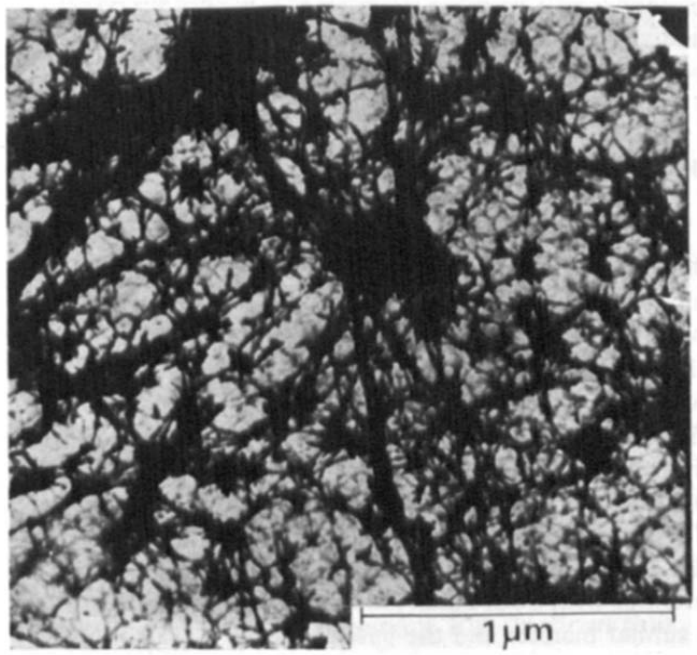

Fig. 4. An electron micrograph of fibres of KAP (F1)-depleted calf thymus histone–coliphage T7 DNA complex.

showed aggregated fibrous structures similar to some of those observed by More and Paul [17]. Georgiev et al. [18] suggest that on adding urea to nucleohistone there is very little dissociation of histone proteins. Therefore solutions of the heterologous complexes were dialysed into 5 M urea and samples for electron microscopy were prepared from these solutions. Stereo-electron micrographs showed reduced aggregation for complexes involving total histone and very much reduced aggregation and the appearance of fibrils, some of which are of the order of 10 nm in diameter, with the complexes involving KAP (F1)-depleted histone. Fig. 4 is an electron micrograph of the KAP-depleted histone–T7 DNA complex used for fig. 1b prepared from 5 M urea solution. The 10 nm fibres can be seen in the figure and in many ways they resemble fibres observed by Bram and Ris [6] from native nucleohistone preparations.

Thermal denaturation profiles for the heterologous histone–DNA complexes at 260 nm using a Perkin–Elmer double beam spectrophotometer showed that the melting temperature of the prokaryote DNA to temperatures in excess of 90°C. These results indicate that there was very little free DNA in the complexes.

Experiments with coliphage $\lambda/\phi 80$ DNA showed similar results to those described above for T7 DNA, and strong low angle X-ray diffraction rings are observed for histone–$\lambda/\phi 80$ DNA complexes.

4. Conclusions

The strongest evidence for the existence of superstructure in eukaryote chromatin is the presence of a characteristic series of low angle diffraction rings. The observations described above of very similar diffraction patterns from complexes of prokaryote DNA with total histone and KAP (F1)-depleted histone shows that a large part of the prokaryote DNA can be induced by histones to assume similar superstructure to that found in the chromatin of higher organisms. This shows with regard to the superstructure giving rise to the characteristic X-ray patterns that histones interact with both eukaryote and prokaryote DNA in a similar manner and the base sequence is probably not an important factor in their interactions.

These studied are being extended to make use of the well-characterised properties of prokaryote DNA's by i) measuring the change in contour length on the binding of histones and ii) to see whether particular histone fractions bind preferentially to AT- or GC-rich regions of the DNA as has been suggested by Clark and Felsenfeld [19].

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