Organisation of subunits in chromatin

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ABSTRACT

There is considerable current interest in the organisation of nucleosomes in chromatin. A strong X-ray and neutron semi-meridional diffraction peak at approximately 10 nm had previously been attributed to the interparticle spacing of a linear array of nucleosomes. This diffraction peak could also result from a close packed helical array of nucleosomes. A direct test of these proposals is whether the 10 nm peak is truly meridional as would be expected for a linear array of nucleosomes or is slightly off the meridian as expected for a helical array. Neutron diffraction studies of H1-depleted chromatin support the latter alternative. The 10 nm peak has maxima which form a cross-pattern with semi-meridional angle of 8 to 9°. This is consistent with a coil of nucleosomes of pitch 10 nm and outer diameter of approximately 30 nm. These dimensions correspond to about six nucleosomes per turn of the coil.

INTRODUCTION

Considerable evidence now supports the many proposals of a repeating subunit structure for chromatin¹⁻⁷. This subunit the nucleosome probably contains two of each histone^{6,8} H2A, H2B, H3 and H4 complexed with about 200 base pairs of DNA^{1,6}. It has been proposed that the DNA component is wrapped around a protein core^{6,7,9,10} and analysis of low angle neutron scatter curves have confirmed this for solutions of monomer chromatin subunits¹¹⁻¹³. These studies are unambiguous. The radius of gyration of the DNA component of the subunit (protein scatter matched by solvent) is 4.90 nm while that of the histone component (DNA scatter matched by solvent) is 2.82 nm; the subunit if considered spherical would have a diameter of 10.2 nm¹². High resolution nuclear magnetic resonance studies of histone complexes^{14,15} show that complex formation involves interactions of structured apolar carboxyl regions leaving the very basic N-terminal regions free and mobile. With individual histones their basic regions are found to complex strongly with DNA^{16,17}. A general model proposed from such studies is that the core of the chromatin subunit consists of a

complex of the structured apolar segments while the very basic segments of histones are complexed with DNA on the outside of the unit⁷. In such a model the basic N-terminal regions of the histones are available for chemical modifications such as acetylation, methylation and phosphorylation.

Of considerable current interest is the organisation of the nucleosomes in chromatin. X-ray and neutron diffraction patterns of chromatin fibres show a series of low angle rings^{7,18,19,20} at *ca* 10, 5.5, 3.7, 2.7 nm which arise from the structure of the subunits and their arrangement in the chromatin fibres. The 10 nm peak which is concentration dependent^{7,19} has been attributed to the basic repeat of the subunits along the chromatin fibril i.e. the spacing between adjacent nucleosomes in linear array^{6,7}. However, whereas it is clear that the 10 nm peak arises from the packing of nucleosomes other arrangements of nucleosomes will give rise to the limited number of diffraction peaks observed for chromatin. Of particular interest are close packed helical arrays of nucleosomes²¹ because they are attractive models for packing subunits into higher orders of chromosome structure. A direct test of such helical arrangements of nucleosomes is whether the 10 nm peak forms a cross-pattern and has maxima on each side of the meridian. If this splitting is small it would appear meridional in X-ray patterns because of disorder in the fibres and the limited specimen to film distances used in X-ray studies.

Neutron diffraction has several advantages over X-rays in studying both the spatial arrangement of protein and DNA in the nucleosomes and the organisation of nucleosomes in chromatin. In addition to the ability to match the neutron scatter of protein or DNA by varying the H₂O/D₂O ratio of the aqueous medium⁷ neutron detection systems are in advance of those for X-rays. This provides the possibility of obtaining absolute intensities of neutron diffraction peaks. Further, with the neutron scattering instrument D11²² at the Institute Laue Langevin large specimen to detector distances can be obtained which allow a detailed study of the distribution of neutron intensities within the envelope of the meridional arc of the 10 nm peak. In previous X-ray diffraction studies it has been shown²³ that specific removal of the very lysine rich histone from chromatin results in an improved definition of the characteristic series of low angle diffraction rings. In seeking an explanation of these diffraction rings we have therefore studied oriented films of H1 depleted chromatin using neutron scattering. In this preliminary communication we report that the maxima of the 10 nm peak are not truly meridional, but form a cross

pattern suggesting a flat helical arrangement of chromatin subunits.

Preparation of Chromatin

Chromatin was prepared from fresh, unfrozen calf thymus glands by the method of Panyim, Bilek and Chalkley²⁴. Slight adjustments to the homogenisation conditions were made to suit the needs of preparing rather larger quantities of material than those originally described. Removal of the H1 histone was achieved using the technique of Bolund and Johns²⁵.

The calf thymus chromatin was dissolved in a buffer of 0.65 M NaCl, 10 mM tris-HCl, pH 7.0 to give a final concentration of 0.75 - 0.80 mg cm⁻³. Bio-Rad, AG 50 W - x2, cation exchange resin, previously washed with the above buffer was added to the chromatin, at a ratio of four volumes of resin per mg, and the mixture stirred for 1 hour at 4°C. The mixture was centrifuged at 3000 x g for ten minutes and the supernatant dialysed exhaustively against 1 mM tris-HCl pH 7.0.

Preparation of Fibres

After an initial concentration of the H1-depleted chromatin, by centrifugation, to 5% - 10% w/v, further concentration was effected by placing the partial chromatin in a teflon trough and passing a rapid stream of cold filtered air over the surface. When the material had reached a tacky consistency it was placed in a sealed tube and allowed to equilibrate for twenty four hours.

The fibres are rapidly drawn by placing the depleted chromatin on the arms of movable callipers and inducing at least an eight fold extension. Drying was continued by again using a stream of cold air.

The original dry fibres were cut into numbers of smaller lengths which were then mounted vertically in a teflon holder. The approximate dry weight of the fibres in each holder was 100 mg. Samples prepared in this way were equilibrated in a moving atmosphere, of the required humidity and D₂O/H₂O composition, for at least twelve hours before measurement.

RESULTS

The first indications that the 10 nm diffraction peak might have intensity maxima slightly off the meridian came from a study of oriented fibres of H1 depleted chromatin equilibrated at 32% relative humidity (r.h.) in 100% D₂O. In these dried films the peak is found at 8.7 nm probably as a result of closer packing of the subunits. Figure 1 shows

FIGURE 1 Oriented fibres of H1 depleted chromatin equilibrated in D_2O at 32% r.h. Uncorrected contours of neutron counts per detector element. The area detector comprises 64×64 elements. Contours at intervals of 100 counts/element from 1200 to 1600 at the 8.7 nm peaks; sample to detector distance 240 cm. Neutron wavelength $0.6 \text{ nm} \pm 9\% \text{ fwhm}$. The arrows indicate a line of detectors with lower sensitivity.

FIGURE 2 Quadrant pattern of contours of neutron counts per element obtained by folding the full pattern about the equator and the meridian to give improved neutron statistics. The contours are given at intervals of 100 neutron counts. Recording conditions as in Figure 1. The level of incoherently scattered neutrons is at 500 counts per element.

FIGURE 3 Quadrant pattern of Figure 2 with a range of contours between 1300 and 1740 counts in 9 steps.

FIGURE 4 Quadrant pattern from the neutron diffraction pattern of H1 depleted chromatin fibres equilibrated at 98% r.h. and 50% D₂O. Ten contours at intervals of 200 counts from 5000 to 4800. Recording conditions as given in Figure 1.

the uncorrected data with contours of equal neutron counts spaced at a 100 count intervals. The arrows indicate a line of detector elements with low sensitivity resulting in distortion in the contours along this line. The 8.7 nm peak is a semi-meridional arc and displays the interesting features that there are intensity maxima slightly off the meridian forming a cross-pattern. Correcting the data for variations in the detector elements and folding the pattern about the meridian and then about the equator to improve the statistics gives the quadrant pattern shown in Figure 2 in which the off-meridional nature of the peak is more marked. The pattern can be further enhanced by selecting a narrower range of contours as shown in Figure 3. The maxima correspond to an angular displacement from the meridian of about 8° . Figure 4 shows the neutron contours for the same fibres highly hydrated at 98% r.h. in 50% D₂O/50% H₂O where the angular displacements of the maxima at 9.2 nm at 9° off the meridian. Similar results have been observed for a second oriented H1 depleted chromatin film.

DISCUSSION

Although additional and more detailed neutron diffraction studies of oriented fibres and films of H1 depleted and of total chromatin are required of the whole series of diffraction rings the observation of off-meridian maxima of the 10 nm semi-meridional arc suggests that it is the first layer line from a helical arrangement of nucleosomes of 10 nm pitch. The angular displacement of 8 or 9° together with a pitch of 10 nm would give a mean radius of the coil of about 10 nm i.e. a hollow coil with an outer diameter of approximately 30 nm. Assuming that the dimensions of the isolated monomer subunits also apply to subunits packed in chromatin then the dimensions of the proposed coil would correspond to approximately six 10 nm spherical subunits per turn. A feature of earlier X-ray diffraction data has been the absence of an equatorial peak corresponding to the lateral packing of chromatin fibrils^{18,20} though such studies have not usually gone far beyond 10 nm. Observations should now be extended to include spacings corresponding to the packing of a coil with outer diameter of about 30 nm. In Figure 1 there is strong equatorial scatter extending to 4.0 nm and which could contain a broad equatorial peak in the region of 25 to 30 nm.

Attributing the 10 nm peak to the off-meridional reflections of a coil of nucleosomes leads to an explanation of the concentration dependence of this peak in X-ray diffraction of chromatin¹⁹ and of the contrast and

concentration dependence of this peak in neutron diffraction studies^{7,11}. The chromatin concentration dependences in H₂O of this peak in both X-ray and neutron diffraction show that with increasing concentration the 10 nm peak goes through zero intensity to reappear in the dry state at approximately 8 nm. However, for chromatin in D₂O with neutron diffraction no such concentration dependence is observed; instead the peak moves steadily from 10 nm to 8 nm with a gradual reduction in intensity⁷. Also with neutrons the contrast behaviour of the 10 nm peak has been investigated and it has been found that with increasing concentration of the chromatin gel the contrast matched position moves to increasingly lower D₂O contents¹¹. The concentration dependence of the 10 nm peak in chromatin in H₂O as observed by X-rays and the concentration dependence in H₂O and D₂O and contrast behaviour in mixtures of H₂O and D₂O as observed with neutrons form a pattern of behaviour which may be explained by an alternation of protein rich regions with DNA rich regions along the direction of the chromatin fibril. Such an alternation was difficult to envisage with a linear arrangement of nucleosomes consisting of a histone core with DNA wrapped around the outside unless DNA was constrained to lie between the nucleosomes. With a flat coil of nucleosomes however, there is a well defined core of structured apolar histone regions alternating with more diffuse DNA regions along the direction of the coil. It is not unreasonable to assume that the DNA regions together with the basic N-terminal regions of histones will be hydrated in preference to the apolar protein core and therefore the amplitude of the alternating scattering density along the axis of the coil will depend on the state of hydration of the DNA and also on the H₂O/D₂O ratio of aqueous medium. In addition if the supercoil of subunits is not maintained in dilute aqueous solution of high molecular weight chromatin the loss of the 10 nm spacing with decreasing concentration¹⁹ can also be explained. The unwinding of the coil of subunits and loss of contact between histone complexes may also result in some Electron Microscope visualisations of beads with spacer regions.

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