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The structure of the chromatin core particle in solution

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ABSTRACT

The shape and size of the nucleosomal core particle from chromatin has been examined by analysis of neutron and X-ray scattering data from dilute solutions. Calculations of scattering for many different models have been made and only one model was able to account for both the X-ray and neutron profiles. This model is an oblate structure with height about 50Å and diameter 110Å. The DNA is mainly confined to two annuli located at the top and bottom respectively of the core particle positioned on the outside of a compact protein core which has a height of about 40Å and diameter about 73Å.

INTRODUCTION

Nuclease dissection of chromatin into well defined subunits permits physical measurements not previously possible on intact chromatin. In addition, the development of low-angle neutron scattering facilities[1,2] and techniques[3,4,5] has created new opportunities for scattering measurements on nucleoproteins[6,7]. We have further demonstrated the potential of the neutron scattering technique in our studies of nuclease resistant chromatin core particles consisting of 140 base pairs of DNA and eight histone molecules[8]. These studies provided conclusive evidence that the DNA is situated on the outside of a central protein core since approximate radii of gyration of 50Å and 30Å were obtained for DNA and protein respectively. We have extended our neutron measurements to higher scattering angles and have collected similar data using X-ray scattering[9]. The higher angle scattering profiles contain new information on the shape of the particle and the relative locations of the DNA and protein within the particle.

To interpret these new data we have calculated scattering profiles for models defined by separate domains of different scattering densities representing DNA, protein, nucleoprotein and solvent. The distribution of protein and DNA in each model was designed to give radii of gyration similar to those previously determined for the core particle[8]. We have considered a series of different models and have been able to find only one model that is

able to satisfy all our scattering data from the core particle. The model[24] suggests the way in which tetrameric histone core protein complexes[10-12], consisting of histones H2A, H2B, H3 and H4, combine to form the octameric core in the core particle. The DNA is located in two loops, possibly turns of a shallow helix, at the top and bottom of an oblate structure. The model is further supported by experimental neutron scattering data from a histone octamer produced by chemically crosslinking isolated histone core protein.

These new results enable us to interpret earlier X-ray[13-15] and neutron diffraction[16,17] studies from intact chromatin.

EXPERIMENTAL PROCEDURES

(a) Preparation and Purification of Core Particles

Two procedures were used to prepare the core particles. The first was that described by Shaw et al.[18], as used in the original radius of gyration studies[8]. The second method involved lysing the nuclei followed by Staphylococcal nuclease digestion of the resultant chromatin. Nuclei from 24 ml chicken blood were lysed in 50 ml 10mM Tris, pH 7.2 made 0.75mM in calcium and 100 units/ml nuclease added. After 10 minutes' digestion at 37°C the chromatin was adjusted to 10mM EDTA and placed on ice. Core particles were purified by centrifugation for 18 hours at 48,000 rpm using a Beckman Ti14 zonal rotor[19] containing a 10-45% w/v sucrose gradient. The 140 base pair particles were selected from the fractionated gradient profile, dialysed into 10mM Tris 0.7mM EDTA, pH 7.5, and concentrated using an Amicon PM10 membrane followed by pelleting into a sucrose cushion. The sample (0.5 ml) was subsequently dialysed for 30 hours against 3 x 100 volumes of 10mM Tris, 0.7mM EDTA pD 7.5 in redistilled D_2O.

(b) Preparation of Crosslinked Octamer

Histone core protein was prepared as described previously[11,20]. A 3 mg/ml solution of the core protein in 2.0M NaCl, 10mM CHES, pH 9.0, was crosslinked using a final concentration of 1 mg/ml dimethyl suberimidate at 21°C for 30 minutes. Precipitated material was removed by centrifugation at 5,000 rpm for 10 minutes using a Sorvall model RC-5 centrifuge and the supernatant dialysed against 2.0M NaCl 10mM CHES, pD 9.0, in D_2O. Polyacrylamide gel electrophoresis was used to demonstrate the octameric nature of the crosslinked complex.

(c) Scattering Measurements

Neutron scattering profiles were obtained using both the small angle diffractometer[1] at the PLUTO reactor of the Atomic Energy Research Establish-

ment, Harwell, as described previously[8], and the D11 instrument of the Institut Laue-Langevin, Grenoble[2]. Data were collected using the Harwell diffractometer for scattering angles of 0.7 to 16 degrees in increments of 0.1 degree. The D11 instrument was used with a 1.71m sample-to-detector distance and with wavelength 4.34Å. The samples were contained in quartz cuvettes of either 5mm or 2mm path length. Scattering from buffer was subtracted from scattering from the solutions of chromatin particles.

(d) Calculations of Scattering Profiles

Calculations of the scattering from spheres were made using the formula from Guinier[21] and profiles were taken from Mittlebach and Porod[22] for uniform cylindrical models. The majority of the calculations for non-uniform cylinders were made using a computer program developed for models in which three coaxial cylindrical domains of different scattering density were considered. The two outer domains could additionally be subdivided such that each domain included a central zone with one scattering density between zones with a different density. Each model was divided into a large number (typically 700) of small elements of equal volume and the co-ordinates of the centres of the elements used to calculate the spherically averaged scattering profile using the expression derived by Debye[23]. For such models the zones representing different molecular components were assigned arbitrary scattering densities relative to a unit scattering density for the DNA. Calculations were made using an IBM 370/135 computer.

RESULTS AND DISCUSSION

The neutron scattering profile obtained from a 5 mg/ml solution of core particles in D_2O, 10mM Tris, 0.7mM EDTA is shown in figure 1. The profile has a broad secondary maximum in the region 35-38Å. A maximum at this spacing has been observed in the neutron scattering from gels and fibres of intact chromatin[16,17] and from dilute solutions of histone core protein isolated in 2.0M NaCl[10-12]. This profile is rather different from the scattering profile obtained from a solution of core particles examined with X-rays. The X-ray profile from core particles[9,38] is very similar to the X-ray scattering obtained from intact chromatin since both contain maxima at spacings of about 37, 27, 22 and 18Å. In some X-ray profiles from intact chromatin gels a well defined maximum is observed at 55Å, but for dilute solutions of core particles the scattering forms only a shoulder near this spacing. A feature of the X-ray scattering profile for particles in dilute solution is the well defined minimum between this shoulder and the maximum

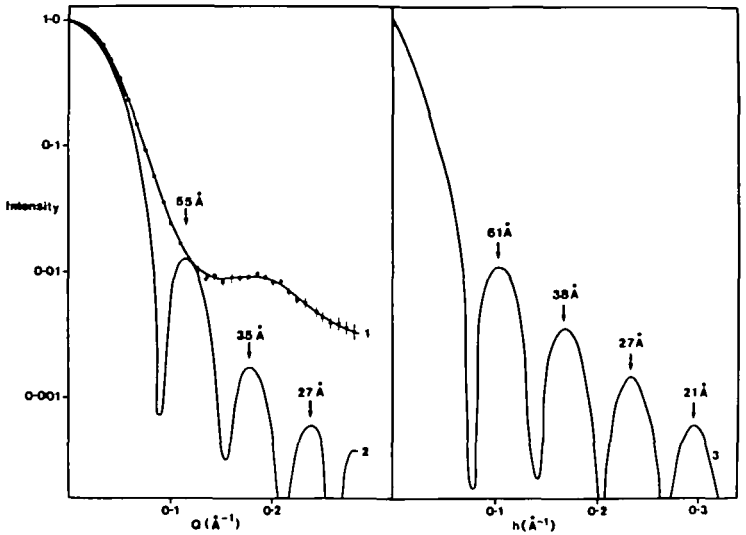

Figure 1: Neutron scattering profile from a 5 mg/ml solution of core particles in D_2O, 10mM Tris, 0.7mM EDTA, pD 7.5 (1) and the calculated scattering profiles for spherical models approximating to an inner sphere of protein surrounded by a shell containing DNA. The radius of the inner sphere is 39.5Å and the outer radius 53Å. Neutron scattering with scattering densities 1.0 and 1.54 respectively for the DNA and protein (2). X-ray scattering with scattering densities 1.0 and 0.49 for the DNA and protein (3). $Q = h = 4\pi \sin \theta/\lambda$ where 2θ is the scattering angle and λ the wavelength.

at 37Å. The differences between the profiles obtained with X-rays and with neutrons can be explained by considering the relative scattering of the DNA and histone. Relative to water, the X-ray scattering density of the DNA is approximately twice that of the histones. Furthermore the DNA is on the outside of the core particle[8]. Therefore, the X-ray scattering profile is largely determined by the overall size of the monomer. In contrast, relative to D_2O, the neutron scattering density of the protein is approximately 1.5 times that for the DNA, and therefore the protein core makes the greater contribution.

We have investigated several types of models in our attempts to explain these scattering profiles. Initially we studied simple structures such as spheres, ellipsoids and cylinders composed of shells of different scattering density but were not able to obtain an acceptable agreement between calculated and experimental profiles. Satisfactory agreement was reached by considering models which are essentially cylindrical but subdivided into more complicated domains of scattering density which accommodate the histone,

DNA (with some histone) and regions of hydration.

We first considered spherical models because the appearance of the chromatin subunit (nucleosome) in the electron microscope both when isolated [25-27] and in chromatin[26,28-30] is circular in profile and thus resembles the bead structure proposed by Olins and Olins [28] and by Kornberg[31]. As an example of the scattering from such models we include in figure 1 both neutron and X-ray profiles for a sphere with overall diameter 106Å having a histone core with diameter 79Å. These dimensions give approximately the correct radii of gyration for the DNA, the histone and the complete particle[8]. Relative to solvent, the scattering densities of DNA and protein were 1.0 and 0.49 respectively for the X-ray calculation (particles in H_2O) and 1.0 and 1.54 for the neutron calculation (particles in D_2O). The agreement with the experimental profile is reasonable for X-ray scattering although the 61Å secondary maximum is not seen experimentally as a maximum but as a shoulder. For neutron scattering the agreement is poor since a series of secondary maxima appear in the calculated profile rather than the single 35-38Å maximum observed experimentally.

Furthermore the calculated intensity in the 36Å region relative to the zero angle intensity is about a factor of ten less for the spherical model than is observed. Since the greater difficulty was found in explaining the neutron data we concentrate here on those calculations. Where a satisfactory fit was obtained between calculated profile and neutron data, for particles in D_2O, we then calculate the X-ray scattering for the same model.

For the non spherical models a computer program which divided the model structure into coaxial cylindrical domains was used to calculate the scattering profiles. The scattering density in the domains could be chosen to give regions corresponding to DNA, nucleoprotein, histone or solvent. Hence it was possible to use the program to study uniform cylinders, approximations to both prolate and oblate ellipsoids and more complicated cylindrical models.

In considering cylindrical models we have examined structures with diameters in the range 70 to 130Å but have concentrated on models with diameters in the range 90-110Å since these give the best fit to the neutron high angle scattering data presented here and with previous data from both radius of gyration determinations[8] and measurements of the hydrodynamic diameter[32]. Initially we calculated the theoretical scattering from coaxial cylinders in which a cylindrical protein core is sheathed by a

cylinder of DNA or nucleoprotein. Figure 2a summarises three calculations of neutron scattering profiles for cylinders of different heights. For cylinders in which the height is about equal to the diameter a series of secondary maxima are obtained in the neutron case rather similar to those for a spherical model. On increasing the height, to give a model approximating to a prolate ellipsoid, the calculated neutron scattering was again in poor agreement with the experimental data. On reducing the height to 55Å the series of maxima are replaced by a single maximum which is in approximately the same position as the experimental secondary maximum. The calculated X-ray scattering profile of a cylinder 55Å high, shown in figure 2b, has a shoulder at 57Å and maxima at 38 and 28Å; these are the main

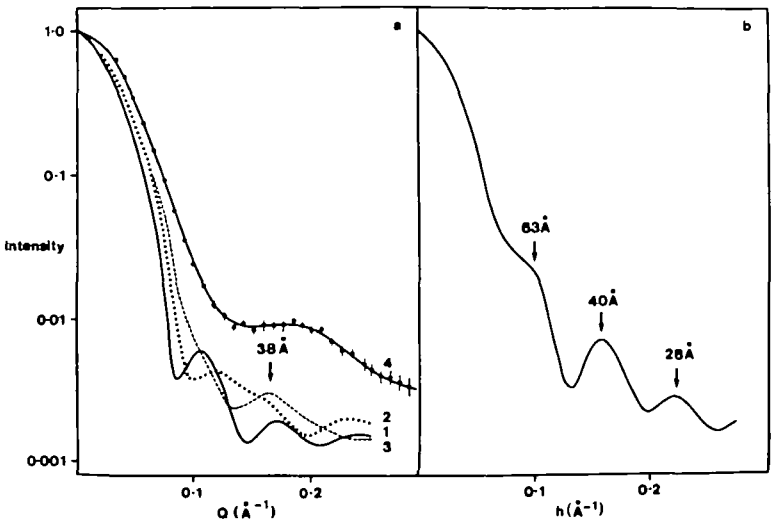

Figure 2:

(a) Calculated neutron scattering profiles for cylindrical models with an inner cylinder of protein radius r_i surrounded by a cylindrical shell of DNA with outer radius r_a. Both inner cylinder and outer shell have height h.

1. h = 90Å, r_a = 50Å, r_i = 30Å
2. h = 66Å, r_a = 51Å, r_i = 30Å
3. h = 55Å, r_a = 54Å, r_i = 31Å
4. Experimental scattering from a 5 mg/ml solution of core particles.

(b) Calculated X-ray scattering profile for a cylindrical two component model with h = 55Å, r_a = 54Å and r_i = 31Å. The DNA and protein were assigned arbitrary scattering densities as for the models in figure 1.

features of the experimental X-ray scattering pattern for core particles in dilute solution. A cylindrical model of 55Å height therefore does account for the positions of both the secondary maximum observed with neutrons and the combination of a shoulder and a series of maxima seen in the X-ray scattering pattern. The major discrepancies between the calculated and observed profiles are the intensity of the secondary maximum with neutrons, which again is too low in the calculated profile, and the relative intensities of the maxima in the X-ray scattering profile. The positions of the calculated maxima for both X-ray and neutron scattering however closely agree with the experimental positions.

The third type of structure that we have considered is an extension of the above cylindrical model with a protein core partially enclosed by cylindrical regions containing both DNA (or nucleoprotein) and water. Possible domains for the DNA relative to the protein core which we considered were a single 20Å high annulus wrapped around its centre, discs of DNA at the top and bottom and two annuli of DNA, spaced by water. Calculations of neutron scattering for these three structures are shown in figure 3. The latter model is the only type of structure which we have found which gives a good fit with the experimental results. In this example the model has an overall height of 50Å and a diameter of 110Å. The protein core is 50Å high and has a diameter of 74Å. Two rings with scattering density equivalent to nucleoprotein are positioned at the top and bottom on the outside of the protein core and are spaced vertically by a 17Å band of water.

Having established that an oblate structure with two loops of DNA on the periphery of a protein core gives the best agreement with the neutron scattering data, the model was adjusted to improve the volumes for the protein and DNA components. Since there is insufficient DNA to fill two complete loops the DNA is limited in our final model to two three-quarter annuli joined by a short segment. This gives an overall DNA length close to that of a 140 base pair DNA molecule. Another feature which we include in the final model is a region of low hydration at the centre of the protein core (scattering density 1.54), compared to more hydrated protein domains on the outside (scattering density 1.08). About 24% of the protein, most probably corresponding to the very basic N-terminal tails[33,34], is in close association with the DNA giving the domain occupied by the DNA and histone tails a scattering density of 1.02. The comparison between the experimental and calculated neutron scattering profiles for the final model and a cross-section showing the distribution of scattering densities is shown in figure 4a. The scattered intensity in the region of the secondary maximum is similar

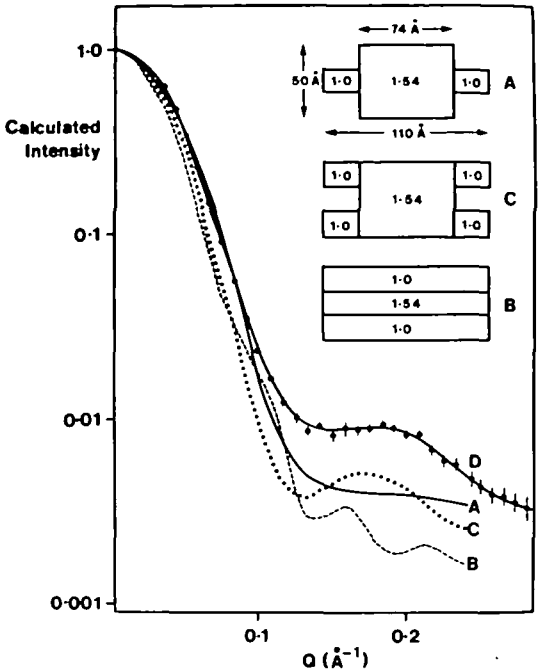

Figure 3: Calculated neutron scattering profiles for cylindrical models in which the domains filled with DNA (scattering density 1.0) are varied relative to the protein core (scattering density 1.54). The schematic cross-sections included are labelled according to the scattering densities of the various domains. Experimental scattering from a 5 mg/ml solution of core particles (D).

in both calculated and experimental profiles. The equivalent calculation for the X-ray scattering profile is shown in figure 4b. A comparison between the calculated radii of gyration and those previously determined is presented in table 1 which also includes data summarising the volume of protein and length of DNA in the model. A schematic diagram of the final model is shown in figure 5.

It should be stressed that the model we propose is largely based on scattering curves obtained using both X-ray and neutron scattering techniques. These data, unlike data from single crystals, are insufficient for the determination of a unique three dimensional structure since the intensities measured are spherical averages. It can be rightly argued that although we have tried many models and only achieved a reasonable fit with one model, this does not eliminate other types of structure not considered. We have however invoked data from hydrodynamic and electron microscopic studies and been limited in our choice of model by the original radius of

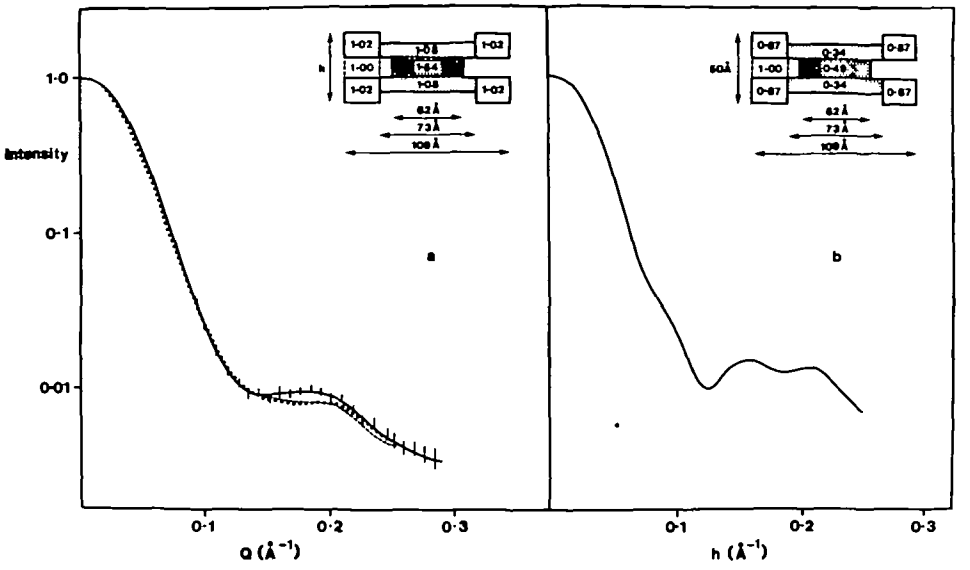

Figure 4: Neutron and X-ray scattering profiles for the 'final' model. The dimensions and distribution of scattering densities are shown in the schematic diagrams which are sections through the model along the cylindrical axis. The domain with scattering density 1.0, between dashed lines in the cross-section, corresponds to a short segment of DNA joining the two three-quarter filled annuli of DNA and protein (scattering density 1.02 and 0.87 for neutrons and X-rays respectively).

(a) Neutron scattering

Experimental profiles as shown in figure 2

Calculated profile with h = 50Å

Calculated profile with h = 53Å

(b) X-ray scattering with h = 50Å

gyration data[8,51]. Working within the constraints imposed by this additional information we believe that we have considered all possible types of structure and since we have found no other model capable of accounting for the neutron scattering profile we are confident that the model we present is correct. The model is limited to a resolution of about 20Å since the data become inaccurate at the higher scattering angles owing to poor counting statistics. For this reason our model lacks detail in that we cannot distinguish between planar loops of DNA or a shallow helix and also we are not able to define precisely the distribution of the histone within the core of the particle. We therefore conclude that the core particle is an oblate structure with a height of about 50-55Å and a diameter of about 110Å,

TABLE 1

	Experimental Result	Calculated for final model, h = 50Å
Radius of Gyration (protein dominates)	30.6 ± 2.0Å[a]	31.5Å[b]
Radius of Gyration (DNA dominates)	50.5 ± 1.4Å[a]	51.2Å[b]
Radius of Gyration	41.1 (± 0.4)Å[a]	41.6Å[b]
Non-hydrated protein volume	132,360(Å)^3[c]	133,373(Å)^3[d]
Length of DNA	476Å[e]	503Å[f]

(a) Data taken from (8).
(b) Calculated using the volume elements for the model scattering calculation weighted with the appropriate densities.
(c) Calculated using the amino acid composition for histones H2A, H2B, H3 and H4 and data taken from Zamyatnin[50].
(d) Calculated from the dimensions of the model.
(e) Corresponds to 140 base pairs of DNA assuming a separation of 3.4Å.
(f) Includes a total length of 440Å in the loops and an additional 63Å joining the loops.

dimensions similar to those described by Wooley and Langmore[35] derived from scanning transmission electron microscopy. In addition we propose that the DNA is largely confined to the top and bottom of the core particle forming two loops separated by a region of water and joined to retain linear continuity of the DNA.

We will describe elsewhere similar calculations for the shape of core protein isolated in 2.0M NaCl, pH 9.0[11,20] which has a radius of gyration of 30Å[17] and is a histone tetramer[10,11,12,17] apparently containing one molecule each of histones H2A, H2B, H3 and H4. The radius of gyration for the core particle under conditions where the octameric protein core dominates the scattering is 30.6Å[8], approximately 1Å greater than the measured radius of gyration of the isolated histone tetramer[10]. In order to demonstrate that an octamer of histones does have the radius of gyration found for the core particle under conditions where the protein dominates the scattering, we have converted isolated tetramers to octamers using dimethyl suberimidate crosslinking. The determinations of molecular weight and the Guinier plot for the crosslinked octamer are shown in figures 6a and 6b respectively. Histones H3 and H4 have been used as reference proteins in the data shown in figure 6a to overcome possible problems due to anomalous behaviour of histones on SDS gels. Aldolase which has a molecular weight more appropriate for this experiment gives the same result. From the neutron scattering data we obtain a radius of gyration of 30.2 ± 0.4Å, agreeing, within experimental

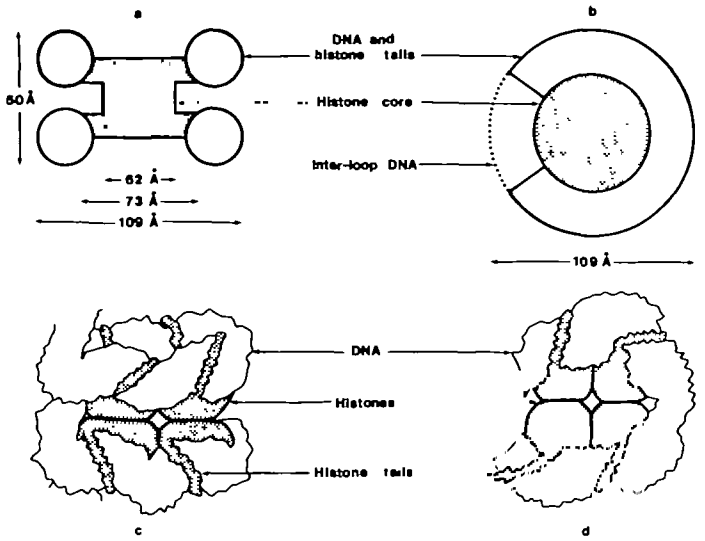

Figure 5: Schematic representation of final model for core particle. Cross-sections showing the domains of the DNA and histones in the model used in the final calculations:

(a) along the axis of the DNA annuli;

(b) perpendicular to the axis of and through the centre of one of the DNA annuli.

Views from the side (c) and looking down the axis of the particle (d).

error, with the radius of gyration (30.6 ± 2.0Å) for the histone core contained in the core particle. The reason for the small difference between the radii of gyration (Rg) of the tetramer and the octamer can be established from a consideration of the relative contributions from the height (h) and radius (r) of a cylindrical model. The radius of gyration for such a cylinder is given by $Rg^2 = \frac{r^2}{2} + \frac{h^2}{12}$. Thus any change in radius makes a greater contribution to the radius of gyration than an equivalent change in height. When two tetramers of the dimensions we have obtained for the isolated core protein[10] are stacked directly on top of each other, the radius of gyration changes by only about one angstrom. The change in radius of gyration would be larger if the tetramers stack without coincident cylindrical axes since the overall width of the protein core would be increased. The small measured difference in radius of gyration between the octamer and the tetramer is therefore consistent with our model for the core particle, having a central compact protein core formed by the stacking together of

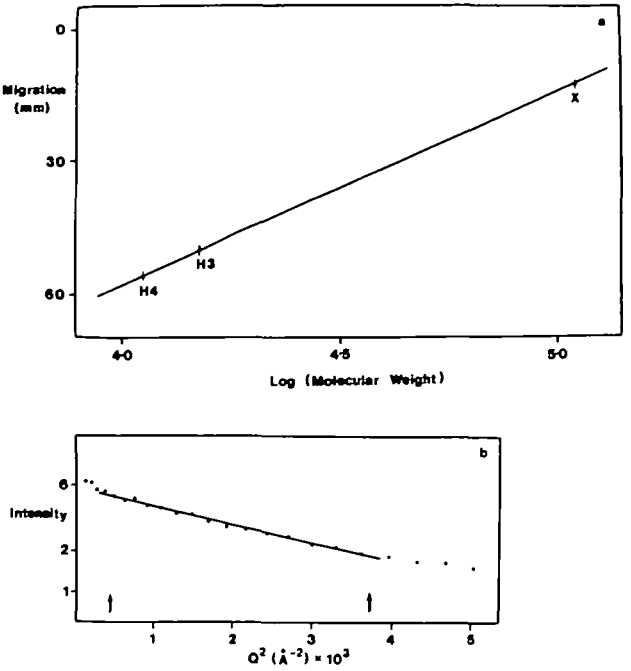

Figure 6: Determination of the molecular weight (a) and radius of gyration (b) of isolated core protein cross-linked with dimethyl suberimidate.

(a) Result of 10% polyacrylamide SDS gel electrophoresis using histones H3 and H4 as markers. The core protein band, labelled X, gives a molecular weight of 110,000 ± 3,000 daltons;

(b) Guinier plot from a 3 mg/ml solution of cross-linked core protein in D_2O, 2.0M NaCl, pD 9.4. Arrows indicate the extent of the data included in determining the radius of gyration using a linear regression program.

two heterologous histone tetramers, to give a diameter of about 70Å and height of 30-40Å. The remaining regions of the histones not included in the compact centre are in the form of N-terminal tails[33,34] which bind to the DNA in the nucleoprotein regions of our model.

The model that we describe is able to explain the appearance of the core particle as visualised in the electron microscope. The diameter is roughly 110Å which would correspond to the core particle lying flat on the grid[29,30,35,36]. In some examples, especially in micrographs published by Varshavsky et al.[37], we notice images which might represent edge-on views of the particle with the DNA (represented as two prominent bands) located at the top and bottom of the particle. Langmore and Wooley have recently made a quantitative study of electron microscope images of the particle[35,36].

They now conclude that the DNA forms two loops around the particle which itself has a height of about 55Å and a diameter of about 110Å. They show that the DNA most likely enters and leaves the particle on one side, a result consistent with the micrographs of Olins et al.[38]. Similar structures have been described by Tsanev and Petrov[39].

Previous X-ray diffraction studies of gels and oriented fibres of chromatin have shown a series of diffraction maxima at 110, 55, 37, 22 and 18Å. One important characteristic of the diffraction data is that the 110Å reflection, which is present in the concentration range 35-55% w/w, decreases in intensity, and is eventually not present at higher concentrations[15] where the 55Å maximum is strong. The origin of the 110Å reflection has not been established. Finch and Klug[40] have interpreted electron microscopic data in terms of a helical model and suggested that the reflection arises from the helix repeat analogous to the original reasoning proposed by Pardon and Wilkins[41] and a similar model has been proposed by Carpenter et al.[42] for very concentrated gels of chromatin. Previously, Kornberg[31] suggested that the repeat distance between beads is about 110Å and that this may be the origin of the 110Å reflection. Evidence for such a densely packed linear array of beads has been provided by Sperling and Tardieu[43] from X-ray scattering measurements.

It is not possible to predict from our data on the core particle how the particles assemble themselves into the chromatin thread since we have no information on the arrangement of the DNA between core particles. If the core particles assemble edge-to-edge along the chromatin thread a fairly uniform distribution of mass would result. Conversely, if the particles assemble top-to-bottom with their cylindrical axes parallel to the axis of the thread, a greater variation in the distribution of mass might be expected. With both types of arrangement for the particles the nucleofilament has along its axis protein rich regions alternating with regions rich in hydrated DNA. Such a repeating structure within the chromatin filament was predicted by earlier neutron diffraction from fibres and gels of concentrated chromatin[16,17]. From these initial neutron studies[17] it was concluded that the chromatin repeat includes a largely hydrophobic region, most probably corresponding to the centre of the core particle, and also that the DNA and protein are to some extent spacially separated along the fibre axis. Similar arguments can be made for helical or solenoidal assemblies of particles where the centre of the chromatin thread is rich in protein and outer regions rich in hydrated DNA. Interdigitation between

nucleofilaments or helices at high chromatin concentrations should produce a strong 55Å maximum corresponding to the stacking distance of core particles within the nucleofilaments or the repeat distance between gyres of the helices. In either case the 55Å maximum represents the height of the core particle.

There is now good evidence that histones remain attached to DNA along regions of active chromatin[44,45] although the conformation of the nucleoprotein in active chromatin may be altered from that of inactive chromatin[46-49]. Our observations that the octamer dissociates into a tetramer after removal of the DNA[9,10,12] suggests that under certain circumstances there might be an intermediate complex possibly containing only one annulus of DNA associated with one histone tetramer. Such a controlled opening of the core particle structure might have a functional significance in that transcription might be permitted without release of histones from the DNA and an easy direct reassembly of the monomer would be facilitated. A more detailed discussion of the assembly of the core particles and implications of the model described here for mechanisms of transcription and DNA replication are presented elsewhere[24].

Finch and Klug (personal communication) have recently determined the structure of the core particle to a resolution of 25Å by X-ray crystallography, and have shown that the particle is an oblate, bipartite structure with dimensions almost identical to those of our final model. Thus the structure appears to be the same both in dilute solution and in crystals.

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