Model studies of chromatin structure based on X-ray diffraction data

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ABSTRACT

Model calculations are presented in order to interpret the X-ray diffraction diagrams given by chromatin gels. It is shown that by taking into account the hydration of chromatin subunits, the problem of calculating the interference function in concentrated gels is greatly simplified. In this way it is possible to fully interpret the influence of concentration on the position and intensity of the various rings present in the X-ray diffraction patterns. The possibilities and limitations of models based on spherical symmetry are also discussed. It is concluded that each chromatin subunit most likely contains three turns of DNA in each 200 base pairs segment surrounding a central protein core. With the method presented here it is possible to test if other models of chromatin based on different kinds of evidence are compatible with the X-ray diffraction data.

INTRODUCTION

The subunit model of chromatin has been substantiated by different types of experiments, as it has been reviewed by several authors (1,2,3). Recently we have obtained detailed X-ray diffraction data from various types of chromatin (4) which can be interpreted according to this model, as we will show in this paper.

SUMMARY OF EXPERIMENTAL RESULTS

Any acceptable model of chromatin should explain all the known X-ray data, which can be summarized as follows:

1st) Chromatin gels are a mixture of a globular and a fibrous structure (4), thus giving rise to an X-ray diffraction pattern which is also a mixture of the patterns due to each structure. The fibrous component often produces an equatorial

spot, but it also influences other regions of the diffraction pattern. In particular, a significant part of the smoothly decreasing intensity ("background") is due to this component. In order to interpret adequately the experimental results, it is essential to separate both contributions. This can be done more easily in H1-depleted chromatin as shown elsewhere (4). In the following we will use the data obtained in this way to interpret the structure of the globular component. We will not study the fibrous component since its significance has already been discussed in detail (4).

2nd) The diffraction diagram at low concentration (less than 20 % by weight) is continuous, with an ill defined shoulder at an equivalent Bragg spacing about 3.7-4.0 nm (4-7). Neutron diffraction gives a similar result, when a sample of isolated chromatin subunits is used (8).

3rd) At intermediate concentrations (30-65 %) a typical series of rings appears, with approximate spacings 5.8, 3.6 and 2.7 nm (4,5,6,9). The exact value of these spacings decreases as a function of concentration, as shown below in Table I. At the same time the intensity of the 5.8 nm ring relative to the other two rings also decreases (4). All these rings have a slight meridional orientation (10).

4th) In the same concentration interval, these rings are accompanied by an additional ring at 11-12 nm. Its intensity decreases significantly as the concentration increases, so that around 50 % concentration it can not be detected in nucleohistone from either calf thymus (9) or from the sperm of different species, whereas in H1 depleted nucleohistone its intensity is low (4).

5th) When the concentration is increased above 70 %, a reversible transition to a dry structure takes place (4,5,6,9). This transition is characterized by the appearance of a very strong ring at about 8 nm, by the disappearance of the rings at about 5.5 and 2.6 nm and by an abrupt shift in the spacing of the ring at about 3.5 nm, which increases slightly. Both rings at 8 and 3.8 nm show meridional orientation (5,10). At the same time the ring at 1.2 nm, due to the second layer line of DNA

is replaced by a ring due to protein at 1.06 nm (9).

6th) Gels obtained from isolated chromatin subunits show nearly identical features, but in this case the 11 nm ring is weaker than in chromatin (10).

MODELS AND CALCULATIONS

Some of the calculations were carried out with the simplified model shown in Fig. 1. The DNA layer was considered to contain also the highly charged and hydrophilic groups of histones, whereas the inner core would contain the hydrophobic histone portions. The hydrated small molecular weight counterions which neutralize the high negative charge of each chromatin subunit (about 230 charges) were assumed to be present in a hydration layer. This layer will shield the strong electrostatic repulsion between chromatin subunits in concentrated gels. The electron density of the DNA-rich layer was taken to be twice the electron density of the protein core, relative to the water layer. In the calculations, the effective electron density of each layer was the difference between its absolute electron density and the average electron density of the sample. In fact this method of calculation is a simplified way to take into account the long range interference function between chromatin subunits in the concentrated gels.

According to this method the average electron density (in

Fig. 1. A simplified model of a chromatin subunit. A central core of protein ($r=4.1$ nm) is surrounded by a DNA-rich shell ($r=5.3$ nm) and a hydration layer ($r=6.5$ nm).

arbitrary units) of the sample was calculated from the expression:

$$\rho_{av} = 4\pi (2 \times 5.3^3 - 4.1^3) / 3fd^3 = 1065 \text{ d}^{-3} \quad \{1\}$$

, where d is the average intersubunit distance in the gel, measured in nanometers, and f is a packing factor which was taken to be 0.9 in order to allow some interpenetration of the hydration layers. Therefore, the effective electron densities of each layer will be:

$$\rho_w = -\rho_{av} \quad (\text{hydration layer})$$

$$\rho_d = 2 - \rho_{av} \quad (\text{DNA-rich layer}) \quad \{2\}$$

$$\rho_p = 1 - \rho_{av} \quad (\text{protein core})$$

The spherically averaged scattering of X-rays by a group of such spherically symmetrical subunits was calculated using the Debye expression, with the appropriate structure factor for the model subunit chosen (11). The calculations were made on a Varian L-100 computer and the curves were plotted using an incremental Dataplotter 240, connected to a hybrid system EAI Pacer 600.

In the second part of this work, calculations were carried out with more realistic models. The DNA component was simulated by a coiled string of tangent spheres of 2 nm diameter and total length about 70 nm (Fig. 2). An inner core

Fig. 2. Some of the shapes of DNA considered in the model calculations presented here. Their crosssections are approximately elliptical of maximum dimensions 7.2 and 10 nm (A and B) or 7 and 12 nm (C and D). In the vertical direction the outer dimensions of the central core are 8.5 (A), 9.5 (B) and 7 (C and D) nm. All of them give similar scattering curves (12).

of protein was simulated by a compact group of tangent spheres. Water was taken into account by spheres of the same size which occupied part of the surface and the intersubunit regions. Each subunit was thus simulated with a compact cylindrical arrangement of tangent spheres of three types. Detailed coordinates of the spheres used will be supplied on request. The scattering curves were calculated as described above. The following densities were taken for each type of sphere: $\rho_w=0$, $\rho_d=1$ and $\rho_p=0.5$. In concentrated gels, the densities were $\rho_w=-1$, $\rho_d=1$ and $\rho_p=0$. Changes in concentration were taken into account by modifying the amount of water present between neighbouring subunits at different intersubunit distances.

Unidimensional Patterson functions were calculated from the experimentally determined intensities I_n (Table I) by the following expression:

$$P(z) = \sum n I_n \cos(2\pi n z) \quad \{3\}$$

, where n is the order of the reflection and z is the fractional coordinate. The factor n is included to correct for the disorientation of the pattern.

RESULTS

A simplified model

In Fig. 3b and in Table I it can be seen that the simplified model shown in Fig. 1 takes into account several of the characteristic features of the experimental results. In particular, the peak at about 11 nm shows a dramatic change as a function of concentration. At the same time the positions of the peaks move as a function of concentration. Their relative intensities deviate significantly from those experimentally determined (Table I) but they could be easily matched just with a slightly more complex distribution of electron density within the model sphere shown in Fig. 1. However this simplified model has a serious drawback. As shown in Fig. 3a, the scattering curve given by isolated subunits has several well defined maxima, as should be expected from the spherical symmetry of the model used. This behavior is in contradiction with the experimental results, which only show a weak shoul-

Fig. 3. Computer drawn scattering curves calculated for the model shown in Fig. 1: (a), isolated subunits in dilute solution; (b), a linear array of three subunits at a mutual distance of 12 (—) and 11 (---) nm; (c), a linear array of three subunits at a mutual distance of 13 nm. In the latter case only the protein cores are considered in the calculations.

TABLE I

Reciprocal spacings (nm^{-1}) and relative intensities of the scattering curves

Approximate weight concentration	Spacings		Intensities	
	30 %	50 %	30 %	50 %
Experimental	.085-.168-.270-.350	.095-.180-.286-.375	2.0-1-.35-.15	.9-1-.55-.40
Simplified model	.081-.181-.274-.369	.093-.188-.281-.373	4.0-1-.48-.22	2.2-1-.59-.29
Realistic model	.093-.181-.271-.328	.096-.191-.287-.349	1.6-1-.50-.39	.82-1-.66-.30

The experimental values reported were obtained with calf thymus nucleohistone depleted of H1 (4)

der at about 3.8 nm under these conditions (4-8). This fact indicates that chromatin subunits are not exactly spherical and, therefore, a model based on spherical symmetry is only useful as a preliminary approximation.

To conclude this section it is interesting to consider the scattering produced by protein alone in this model, shown

in Fig. 3c, since this situation occurs in neutron scattering experiments (13). The relatively large intersubunit distance, compared with the size of the protein core, gives rise to a well defined peak, which is the most prominent feature of the experimental results under conditions in which the contribution of protein to the scattering curves is enhanced (13). The position of this peak is directly related to the intersubunit distance.

A more realistic model

Recent results obtained in our laboratory (10) indicate that all the scattering rings show a certain degree of meridional orientation. This fact had already been observed by other authors in some of the rings (5,13,14). Therefore it appears that neighbouring subunits have a tendency to align themselves close to the axis of the fiber. If all the rings had a truly meridional origin, an unidimensional Patterson function based on their relative intensities would reveal important features of the electron density distribution within each subunit. The results obtained from such calculation are shown in Fig. 4. As a result of the relatively high intensity of the peak at about 5.5 nm, a clear electron density peak appears at the center, which indicates that a considerable amount of material occurs at mutual distances which are about half the intersubunit spacing. With this information tentative subunit shapes can be drawn, as shown in Fig. 2. All of them have very similar scattering curves, as it has been shown elsewhere (12). Model A and B are distorted coils. In A the pitch of the helical region is 3 nm, whereas in B it is 4 nm. Model C has the DNA organized as the seam in a tennis ball. Model D is a two turn distorted coil. Model C can be converted into D just by changing the position of a small part of the DNA. All the models have an approximately elliptical crosssection, because if it is assumed to be circular, strong peaks appear in the scattering curve calculated for isolated subunits. Furthermore, the coils have been distorted in such a way that approximately flat turns are placed at both edges of each subunit, so that their projections on the axis of the particle produce electron density maxima at about half the

Fig. 4. Unidimensional Patterson functions calculated from the intensity values given in table I. The weight concentration is 30 % in A-C, whereas in B-D it is 50 %. Four orders are considered in C and D, but in A and B the third order (3.7 nm) is excluded for reasons explained in the text.

repeat distance, as indicated by the Patterson functions shown in Fig. 4.

The scattering curves calculated for model A are shown in Fig. 5. Isolated subunits in water show a single shoulder at about 3.5-4.0 nm (Fig. 5a) as it is found experimentally. When an isolated subunit is transferred to a concentrated gel, the scattering curve (Fig. 5b) changes significantly, due to the increased average density of the sample and to the presence of a hydration layer. Under these conditions, a peak may appear in the 10-20 nm region. Its eventual appearance, position and intensity are strongly dependent on small changes in the degree of hydration and average density of the sample, so that this peak does not appear to be directly related to the 11 nm peak which is experimentally observed. Groups of two subunits give the scattering curves shown in Figs. 5c and 5d. Quantitative values for these two curves are shown in Table I. It can be seen that there is reasonable agreement with the experimental values, in particular in the fact that the spacings and intensities show changes with concentration which are in the right direction. The quantitative agreement is not absolute, but the models used could be easily optimized by

Fig. 5. Scattering curves calculated for the model shown in Fig. 2a: (a) an isolated subunit in water; (b) an isolated subunit in a concentrated gel; (c) a group of two subunits displaced 12 nm in the vertical direction; and (d) a group of two subunits displaced 11 nm in the same direction.

changing slightly the dimensions and electron density distribution within the subunits. However the limited amount of experimental data available do not warrant such an optimization.

An important parameter in the calculations is the relative position of the subunits. The curves shown in Figs. 5c and d were calculated with the subunits placed one on top of the

other. Instead, if they were placed side by side, the agreement with the experimental results was lost. It appears therefore that there is a certain degree of preferential orientation in neighbouring chromatin subunits. Most likely this preferential orientation occurs in directly connected subunits, although other intermolecular forces, such as histone-histone interactions, may play an important role in determining such orientation.

The dry state of chromatin

At high concentrations, the scattering pattern of chromatin is characterized by a well defined strong peak at about 8 nm. The presence of this strong peak indicates that at this level of resolution there is a clear segregation of regions with very different electron densities, in our case protein-rich and DNA-rich regions. It is unlikely that water is segregated in regions of this size, since at these high concentrations most of it will be involved in the short range solvation of the electrically charged groups. In principle these segregated regions may have very different shapes, such as layers, alternate segments of a rod (10), etc. An interesting possibility to consider is that the protein and DNA rich regions are arranged as concentric spheres, as shown in Fig. 1. In this case no hydration layer will be present and the scattering curves should be calculated with a negative value for the effective electron density of the protein core and a positive value for the DNA rich shell. The absolute values of these effective electron densities should be chosen in such a way that the average effective electron density of each particle is zero. The scattering curve calculated for such a particle is shown in Fig. 6. A strong maximum at the experimentally found position appears, with a series of small intensity secondary maxima. Chromatin samples only show a shoulder at about 3.8 nm, which indicates that the subunits can not be exactly spherical. Nevertheless, a quasi-spherical model seems adequate to explain the results experimentally found.

The peak at 3.5-4.0 nm

The origin of the peak or shoulder which appears at these

Fig. 6. Scattering curve given by a sphere such as the one shown in Fig. 1, but with no hydration layer. The radius of the protein core is 3.97 nm and the outer radius is 5 nm. The effective electron densities of the protein and DNA rich regions were taken to be equal, but with opposite signs. The two first maxima appear at .118 and .259 nm⁻¹.

spacings has to be considered in detail, since it is found at all concentrations (4-9). In neutron scattering experiments (13) a peak also appears in this region. It is interesting to note that when the contribution of protein is enhanced, only this peak and the strong peak at about 10 nm are observed, without any trace of the peaks at about 5.5 and 2.7 nm. From these observations we conclude that the peak in this region reveals an outstanding feature of the transform of individual chromatin subunits. For this reason we have not included the contribution of this peak in some of the curves shown in Fig. 4.

The origin of this peak may be traced to either a shape factor, as it occurs in ellipsoids which are moderately asymmetric (15), or to an internal repeat inside the subunit, such as the distance between fragments of the DNA component. The fact that it is also observed when the contribution of protein is enhanced (13) does not exclude the latter possibility, since under these conditions the DNA is equivalent to voids in the chromatin subunit, and their spacing may contribute significantly to the observed neutron scattering curves. As a matter of fact, we have observed (10) a meridional or quasi meridional orientation of this ring, an orientation which is very prominent around 60 % weight concentration. This observation gives

support to a distribution of DNA in the chromatin subunit similar to the one shown in Figs. 2a and b, with a distance between neighbouring DNA turns close to 3.5-4.0 nm.

In the dry state of chromatin this ring is also present, but its spacing moves to higher values (4). This observation may be simply due to a sampling effect, as a second order of the prominent peak at about 8 nm. However it can not be excluded that under these conditions this peak has a different origin, since in the dry state DNA is significantly distorted (9). As a matter of fact, the spherical model shown in Fig. 6 has a second order peak with the correct intensity in this region.

DISCUSSION

The prominent changes of the intensity of the scattering rings with concentration are one of the most characteristic features shown by chromatin gels. This effect is most noticeable for the ring at about 11 nm, but significant changes are also observed in the rest of the rings. In this paper we have shown how this effect can be interpreted as a result of the existence of a hydrated region around chromatin subunits, together with changes in the distance between subunits as the concentration increases. However the limited data presently available do not allow an unequivocal interpretation of the results in terms of a unique shape of the chromatin subunit. As the concentration changes, the scattering curves are also influenced by changes in the shape and mutual orientation of chromatin subunits (which must occur however small due to the fact that they are connected by DNA) and by changes in the number of subunits coherently scattering. These effects have not been considered here, since they would involve arbitrary assumptions.

In a previous work (12) we had investigated the influence of the shape of the DNA component on the scattering curves. However at that point we were faced with the difficulty of taking into account the changes in the average electron density which occur as a function of concentration. In this paper we have shown how this difficulty can be solved by calculating the scattering of subunits which have an average electron

density equal to the "solvent" electron density. This is equivalent to assume that there is very little or no long range order of neighbouring chromatin subunits not directly connected. It should be noted that the fibrous component of chromatin (4), is also part of the "solvent" in which individual subunits occur in concentrated chromatin gels. In some instances this method of calculation produces peaks at very low spacings (Figs. 5b and 5d), which are related to the overall size and shape of the coherently scattering regions. The peaks experimentally observed by Bram et al (16) in this region could be explained in this way.

In the first part of this paper we have shown how the experimental results can be interpreted with a model which has spherical symmetry (Fig. 1). We have determined its dimensions in order to achieve optimal agreement with the experimentally observed scattering peaks (Table I). It is remarkable that the dimensions chosen coincide with those obtained by Pardon et al (17) using chromatin subunit particles, although their particles only contained 140 base pairs of DNA. In favour of this model is the fact that the most hydrophilic histone residues (basic, acidic, serine, threonine) will occur in the surface of the particle. The occurrence of these residues, which have a strong scattering power, together with DNA, will give rise to an outer layer of high electron density which may well have an approximate spherical shape.

The fact that the particles are formed by a low density protein core surrounded by an electron dense shell also explains the very different behavior of the 11 nm peak in neutron and X-ray scattering experiments. In the first case, when the contribution of protein is enhanced, this peak maintains its intensity throughout the whole concentration range (13), since given the comparatively small size of the protein core, the sampling effect due to the intersubunit distance always occurs in the large central maximum of the transform of the protein component. On the other hand, in the X-ray experiments, in which the scattering intensity is mainly determined by the contribution of DNA, as the intersubunit distance decreases, the intensity of the peak at about 11nm sharply de-

creases as we approach the edges of the central peak of the DNA transform, which is narrower than that of protein due to the larger size of the DNA region in the chromatin subunits.

The strongest limitation of this simple model is due to the fact that it is unable to explain the scattering behavior at low concentrations, where only a shoulder at about 4 nm is observed. A possible explanation would be that chromatin subunits are distorted from spherical symmetry at low concentrations, perhaps by extensive hydration of some of the DNA. The peak observed under these conditions could then be due to the shape and structure of the hydrophobic protein core with a limited amount of tightly associated DNA. However this possibility appears to be in contradiction with the radius of gyration determined by Pardon et al (17) for the DNA component.

The spherical model we have just discussed has some similarities with the calculations recently presented by Carlson and Olins (18). However they did not consider the presence of a hydration layer. As a result their model requires a large number of subunits in register (at least ten) in order to develop a distinct peak at 11 nm. Furthermore the relative intensities of the different peaks deviate significantly from those experimentally determined and no account is given of the influence of concentration on the X-ray diffraction pattern. These authors suggest (18) that the electron density distribution within the v body is an important consideration in order to improve their results. The calculations presented here show that this is indeed the case.

In the second part of this paper we have considered the possible shapes for the DNA component. By choosing a slightly asymmetric shape for the subunit, it is then easy to explain the presence of a single peak at low concentrations (Fig. 5a). The peaks which appear as the concentration increases are produced by a sampling effect of the intersubunit distance. In a previous work we had already shown (12) that there is a whole family of DNA configurations, with two or three turns of DNA per subunit, which give very similar scattering curves. In order to obtain agreement with the experimental results, in particular with the prominent intensity of the 5.5 nm peak,

it is essential that there are at least two regions in each particle with a high effective electron density, a fact which limits the choice of possible shapes for the DNA component. From the radii of gyration determined by Pardon et al (17) it appears that three turns are more adequate to accommodate 200 base pairs of DNA. As a matter of fact these authors suggest two turns for the 140 base pairs segment of DNA present in their chromatin subunit particles, which is equivalent to our proposal. The orientation effects of the 3.7 nm peak give also some support to this suggestion. However, it is not possible to decide which is the actual path followed by a DNA molecule in a subunit. It may follow a helical path (Fig. 2a) or it may form a zig-zag or more complicated shape around the protein core.

Although the experimental results are compatible with a subunit particle containing three turns of DNA, this can not be considered as actually proven. As we have discussed above, the experimental results as well as the method of calculation used are limited. In particular no account has been taken of the changes in scattering power in different regions of the protein. However, the method of calculation used here is suitable to test some of the models which have been recently suggested. For example, we have calculated the scattering curves given by the hairpin model of Van Holde et al (19) and by the solenoid model (20) and they deviate very significantly from those experimentally determined. On the other hand, the model suggested by Walker and Hyde (21), with some distortion, is equivalent to the model shown above in Fig. 2d.

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APPENDIX

After submitting this paper, several related works have appeared (1a-4a) on which we wish to comment in this appendix. First of all we must stress that the scope is different. In our paper we are concerned with the basic features of the X-ray diffraction patterns given by nucleohistone. We show how the

experimental results can be interpreted taking into account the electron density distribution within a subunit and the interaction between groups of two neighbouring subunits. In our paper we do not draw any conclusion on the spatial distribution of chromatin subunits, they might be arranged in linear strands, coiled (3a), in a zig-zag pattern, etc. Actually, the poor orientation of the patterns presently available, does not allow to draw any safe conclusion in this sense. For this purpose it is necessary to study in detail the slight orientation of some of the scattering rings, a task in which we are presently engaged (10). In this sense, the experiments reported by Carpenter and collaborators (1a) offer a great interest. As pointed out by these authors, their results are consistent with a coil of nucleosomes of pitch about 10 nm. However other interpretations are possible and in no way they constitute a sure sign that they originate from a helix (4a). The fact that the 10 nm peak is slightly off the meridian may be a feature of the oriented transform of an individual subunit, so that these results are not necessarily inconsistent with a linear arrangement of nucleosomes. Another possibility is that nucleosomes are arranged in a zig-zag fashion, a spatial arrangement which would also result in a splitting of the 10 nm peak. Actually, the tendency of nucleosomes to show lateral association (5a) gives some support to this possibility.

The pairs of subunits coherently scattering which we have considered in our paper may be directly connected by DNA, but this is not necessarily so. Histone-histone interactions and other intermolecular forces may result in a side by side organization of nucleosomes, which could very well originate coils of variable diameter with a pitch close to 10 nm, as suggested by Finch and Klug (3a). Since the suggested diameter for the coils is variable, the scattering diagram of such an arrangement of nucleosomes will be dominated by the interaction between neighbouring subunits, placed at a distance which practically coincides with the average pitch, and by the electron density distribution within each subunit, so that the methodology presented in our paper is equally valid to analyze the main features of diffraction by such a system. As a matter of fact, if a coiled arrangement of nucleosomes in the samples used for X-ray diffraction is substantiated, then our work shows that DNA folds must be oriented rather perpendicular than parallel to the axis of the coil, so that the schematic path drawn by Finch and Klug (3a) in their Fig. 7 can not be correct.

Finally we would like to point out that electron micrographs show that the diameter of chromatin fibers may be very variable in different systems (4a, 6a) and is also influenced by the ionic medium (3a, 7a), so that different types of coiled or folded arrangements certainly occur in vivo, perhaps similar to those suggested by Finch and Klug (3a) or by Carlson and Olins (18). However the arrangement of nucleosomes in the samples used for X-ray diffraction may be different and it probably also depends on their concentration. We must conclude that the overall spatial arrangement of nucleosomes is still an open question.