Chromatin very small angle neutron scattering: further evidence for a 30 nm diameter super coil in dilute solutions

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ABSTRACT

Intact chromatin, chromatin minus histone H1, and nuclease digestion fragments have been studied by very small angle neutron scattering. The results are not consistent with a straight chain of nucleosomes and require the presence of a higher order coiling in monovalent salt solutions. The data are interpretable by a structure having a cross section radius of gyration of 8.5 ± 1 nm, which suggests an outer diameter for a coil of nucleosomes of 27 ± 3 nm.

INTRODUCTION

A wealth of evidence supports the existence of a subunit structure for chromatin (1-5). The relative arrangement of these subunits or nucleosomes along what has been called the chromatin unit fiber (6) is of general interest. Small angle neutron scattering from dilute solutions of chromatin in monovalent salt exhibited scattering intensity maxima at equivalent Bragg spacings of ca. 40 nm (7,8). This was one of the first indications that the higher order arrangement of nucleosomes was a regular supercoil. Since the DNA secondary structure is already wound in the nucleosome to a higher ordered state, we called this first coiling of nucleosomes the quaternary structure (8). A super helix with a pitch of ca. 40 nm and outer diameter ca. 30 nm with 4 to 8 nucleosomes per repeat was found to agree best with electron micrographs of freeze-fractured hydrated chromatin (9,10). Such a loose coiling could be compatible with recent hydrodynamic and light scattering studies on chromatin multimers in dilute solution (11); however, these results were not consistent with a rigid rod of nucleosomes.

Supercoils with the same number of nucleosomes per turn and outer diameter have been proposed for the structure of chromatin at very high concentrations in fibers (12,13) and in the presence of divalent ions (14). But a doubling or fold back looping of the unit fiber has also been suggested to explain this structure (6,15). In any case, it is generally agreed that

the structure in isotropic solutions of monovalent salts is much less compact (11,14,12,15).

Previous X-ray scattering studies of chromatin have been limited to less than 50 nm Bragg spacings (16,17) by classical X-ray diffractometer design. In this report, we present the neutron scattering at two to four times larger spacings. We have studied the neutron scattering from a variety of preparations in different salt and H₂O-D₂O mixtures. In this first article in a series on chromatin solution scattering, we show that the scattering from these chromatins is in accord with a structure having a cross section radius of gyration of 8.5 ± 1 nm and a mass per unit length of $40,000 \pm 10,000$ Daltons/nm. More precise values for these parameters and higher resolution data will be given in other articles.

METHODS AND MATERIALS

Small angle neutron scattering. Kratky and Porod (18) showed that the scattering from isotropic solutions of elongated particles will obey, in a certain angular region*, the following relation :

$$\log [I(h) \times h] = \log(I_0) - \frac{h^2 R_0^2}{2} \quad \{1\}$$

where $h = \frac{4\pi}{\lambda} \sin \theta/2$; λ = the wavelength of the radiation and θ the scattering angle. I_0 is proportional to the mass per unit length (M/L) and the absolute intensity incident on the solution. When the absolute intensity is known, M/L can be determined. If a plot of $\log [I(h) \times h]$ against h^2 is linear, so that $I(h) \times h$ falls by at least two fold, R_0 , the cross section radius of gyration, can be obtained from the slope. R_0 is a measure of the radial distribution of mass in the particle (18).

Experiments were carried out with the D 11 diffractometer built by K. Ibel (19) using a $\Delta\lambda/\lambda$ of 9 % at the Institut Laue-Langevin in Grenoble. Due to the 30 cm radial dimensions of the detector, scattering curves must be obtained in discrete steps using different wavelength and detector-sample distances. The various diffractometer settings employed are given in the figure captions. For each experiment more than 5,000 real chromatin counts were obtained at each radial position. The radial sensitivity of the multi-detector was corrected for by reference to a long run on H₂O.

Chromatin samples for the results shown here were obtained from fresh calf thymus or rat liver using the method of Panymin et al. (20), except that

* This range must satisfy $h \times R_0 \gtrsim 1$ and $h \times \text{particle length} \gg 1$.

nuclei were dispersed by gentle stirring in 1 mM NaCl. Histone H 1 was removed from some preparations by 650 mM NaCl (21). The chromatin preparations have been carefully characterized by us and others with neutron and X-ray scattering (8,13). Electron microscopy shows that nucleosomes are contiguous in our chromatin (16,9). Nuclease digestion fragments were obtained from rat liver and calf thymus nuclei using the digestion conditions described by Noll et al. (22). After purification on a Biogel 5 m column, the average length of the fragments seen in the electron microscope was about 200 nm.

RESULTS AND DISCUSSION

Cross section scattering. The very small angle neutron scattering from the various preparations so far studied are linear over the range $.25 < h^2 < 2.5 \times 10^{-4} \text{ (\AA)}^{-2}$ in the $\log [I(h) \times h]$ against h^2 plots. (See figures 1 and 2). When the scattering obeys equation {1} over at least a two-fold range of $I(h) \times h$, it is customary to assume that it is due to cross section scattering from fibrous regions in the sample. It is therefore reasonable to apply equation {1} to our data. As can be seen in figures 1 and 2, all of the plots would be consistent with a slope equivalent to a R_0 of 8.5 nm within the experimental error of 1 nm. Similar slopes are obtained in the scattering from chromatin with and without H 1 in 1 mM NaCl and from 200 nm digestion fragments.

The salt concentration does not seem to be very important in our experiments. For example, chromatin fragments in D_2O containing 1 mM, 10 mM and 650 mM NaCl yield similar R_0 . Likewise, chromatin concentrations below 5 mg/ml do not significantly effect R_0 , but at greater concentrations R_0 decreases. The results have not been corrected here for wavelength or instrumental smearing effects, as we have found that the error introduced is less than 10 %.

Aggregation is considerably reduced by removal of H 1, and in 650 mM NaCl, yet the cross section scattering of total and H 1-depleted chromatins are similar. Electron micrographs of freeze fracture replicas of the solutions studied show widely separated unaggregated fibers (9). In addition, 200 nm nucleosome nuclease digestion fragments obtained by the method of Noll et al. (22) also have an R_0 of 8.5 nm. Finally, we shall show that the absolute intensity derived from the 8.5 nm line is similar to that expected from a loose coil of nucleosomes. Still, some aggregation may be present and may cause the rise in intensity at the very smallest angles seen in figure 1.

If further information is available, we can make certain deductions about the external diameter from R_0 . It is generally accepted that the chromatin

Figure 1 : Examples of very small angle neutron scattering from calf thymus chromatin : (a) after H1 extraction (5 mg/ml in 650 mM NaCl) obtained with a 40 m detector-sample distance and .54 nm neutrons; (b) total chromatin (4.5 mg/ml in 1 mM NaCl) with a 10 m detector distance and .96 nm neutrons; (c) theoretical scattering from a super helix of 30 double coil nucleosomes having a 42 nm pitch 7 nm nucleosome center radius and a 7 nm axial translation. A line corresponding to $R_0 = 8.5$ nm is drawn with all curves and fits the data within the experimental error.

fibers are elongated chains of nucleosomes (1-5), whose hydrated diameter is between 10 and 15 nm (3,4,9). The cross section radius of gyration of a nucleosome in a fiber (R_n) is then between 4 and 5 nm. That R_0 is about two fold larger than R_n requires that the nucleosomes be folded in a higher ordered structure in our monovalent salt solutions. The parallel axis theorem tells us that $R_0^2 = R_n^2 + r_s^2$: where r_s is the radial distance of the nucleosome centers from the long axis of the super structure. It can then be calculated that a coiled chain of nucleosomes, each 10 nm in diameter, will have an outer super structure diameter of 25 nm, while a coil of 15 nm nucleosomes will have an external diameter of 29 nm. Thus, our results are compatible with a super coil having an external diameter of 27 ± 3 nm.

Comparison to a super coil model. A complementary approach to treating the small angle scattering is to calculate the scattering with the Debye equation (23) for a model derived from other evidence. Support is provided insofar as the theoretical curves agree with the data. The presence of scattering maxima at 40 nm (7,8) and electron microscopy of frozen hydrated chromatin replicas (9,10) have led us to propose a super coil of about 40 nm pitch and 30 nm outer diameter with 4 to 8 nucleosomes per repeat. (Nearly all published electron micrographs obtained on dehydrated chromatin from monovalent salt solutions exhibit loosely coiled fibers (14,3,4,6,16. See for example the "zig-zags" of

Figure 2 : Small angle scattering taken with a 5.4 m sample detector distance : (a) rat liver chromatin nuclease digestion fragments (1.8 mg/ml in 1 mM EDTA pH 7, D₂O); (b) total calf thymus chromatin with a 10 m collimation setting (4.5 mg/ml in 1 mM NaCl, D₂O); (c) calf thymus chromatin (4.5 mg/ml, 650 mM NaCl, H₂O); (d) the theoretical scattering from the super helix of 30 double coil subunits described in the text.

Oudet et al. (24)). Concerning the nucleosome substructure, electron micrographs of freeze fractured chromatin replicas show dense cores surrounded by peripheral coils which join neighbouring nucleosomes (9,10,25). The outer diameter of the peripheral coil is 14 to 15 nm, and it has a contour length of 30 to 40 nm. A corresponding "double-coil" nucleosome structure was suggested (9,10) and found to agree with the neutron scattering from 180 base pair nucleosomes (25). The basic feature of this model is that there are two orders of coiling with opposite handedness per nucleosome. In the superhelical model employed in our calculations, the dense inner coils are in contact with

the peripheral coils of their neighbour (see figure 3).

The scattering from this quaternary super coil of double coil subunits was calculated as previously described (8,25). As can be seen in figures 1 and 2, a good accord with the very small angle data is obtained. However, we must point out that the very small angle scattering at spacings greater than 30 nm is not very dependent on the nucleosome ultrastructure. Yet we have found that a double coil nucleosome model (9,25) gives a better agreement with the higher resolution data than other published models.

The theoretical scattering at the smallest angles is somewhat dependent on the length of the model. Calculations with 30 or more nucleosomes yield a graphical R_0 in agreement with that expected from the coordinates, but shorter models yield a smaller R_0 .

At larger angles, corresponding to about 40 nm, the experimental scattering exhibits a change or an inflection in curvature. (Multiplication of the intensity by h shifts the shoulder to 35 nm as in figure 2). We had previously made a reasonable supposition that it results from the contribution to the scattering of the first order Bessel function of a super helix with a 40 nm pitch (7,8). The 40 nm maximum is weaker than that theoretically expected from a regular super coil, which may be suggestive of some non-uniformity in the super coiling. Imperfections in the collimation and monochromatization also smear somewhat the experimental maximum.

An approximate M/L. The neutron scattering can be roughly put on an absolute scale by matching the scattering at larger angles to that of the X-ray data (16). (A more precise M/L will be published elsewhere.) Between 35 and 15 nm both the neutron and X-ray scattering in H_2O have slopes corresponding to an R of $5.0 \pm .2$ nm. The intercept of the 8.5 nm neutron scattering line is $1.9 \pm .2$ times that of the 5.0 nm line which gave an M/L of 19,000 to 22,000 D/nm (16,9). In this manner we find that the M/L from the intercept of the R_0 line is $40,000 \pm 10,000$ D/nm for total chromatin. This value can be directly compared to that of the super coil model in order to test our results. In the model, six nucleosomes of 280,000 Daltons molecular weight are found in a repeat of 42 nm : thus $M/L = 40,000$ D/nm.

General remarks about interpretation of chromatin solution scattering. It was stressed by Bram and Ris (16) that the scattering from a chromatin solution does not, in general, provide detailed structural information. "Additional information is available from the X-ray scattering to the extent that independent evidence justifies treating the sample in terms of at least a partially defined model" (16). Our freeze fracture electron microscopy carried

Figure 3 : A schematic representation of a possible higher order super coil which would agree with our neutron scattering and electron microscopy results. The nucleosome structure was proposed elsewhere (9,10,25) to contain a peripheral DNA coil 14 to 15 nm in diameter about an inner coil of 4 nm pitch and 8 nm diameter. Since the inner coil is tightly bent it is quite possible that the approximately 80 base pairs therein are kinked. Central histone cores are shaded and are in contact with their neighbour.

out under identical chromatin and ionic concentrations (9,10) and the presence of secondary maximum near 40 nm (7,8) provides, we believe, justification for a treatment of the very small angle scattering by a super coil type model. That the structural parameters obtained by this treatment equals that found by electron microscopy furnishes further support.

In addition to the cautionary remarks above, the presence of a super-structure in dilute salt solutions makes analysis of the scattering at spacings less than 40 nm much more complicated than some workers have assumed (17). We had previously cautioned (16) that, without further evidence, the assumption that a rigid rod structure is present can only give a minimum value for the actual mass per unit length (M/L) and a rough idea of the actual cross

section radius. Moreover, the earlier X-ray scattering study (16) showed marked changes at ionic strengths less than 1 mM; consequently, the superstructure may be different at the 0.2 mM salt conditions employed in the other work (17).

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