CHROMATIN SUBUNIT SMALL ANGLE NEUTRON SCATTERING: A DNA RICH COIL SURROUNDS A PROTEIN-DNA CORE

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Received June 28, 1976

SUMMARY. The small angle neutron scattering radii of gyration of 185 base pair subunits have been determined in H_2O and D_2O. These values suggest that the outer diameter is 120 to 150 Å. The results are not consistent with models in which all of the DNA is in an external shell. The neutron scattering profiles are in good agreement with a model based upon freeze etching electron microscopy (4) having two concentric coils of DNA with 80 Å and 150 Å external diameters.

INTRODUCTION

Chromatin has been shown to contain regular repeating DNAase resistant subunits (1,2) which may comprise up to 85% of the total mass (3). However, the structure of chromatin and these subunits is not well known. The mass per unit length of calf thymus chromatin determined from our X-ray scattering is 2100 daltons/Å (4). This requires that the DNA be contracted 5.1 fold in chromatin and 5 to 7 fold in subunits. Freeze etching electron microscopy of hydrated chromatin gives a diameter of 150 Å for chromatin (4) which is somewhat larger than that of dehydrated material. These micrographs also suggest that the DNA in chromatin is wound in two concentric coils of 150 Å and 80 Å outer diameter (4).

Neutron scattering studies on chromatin (5,6,7) and 140 base pair subunits (8) have been shown to provide very

useful structural information. Subunit models in which all of the DNA surrounds a protein core were published by other groups (7,8). This report presents the neutron scattering from 185 base pair subunits. The data are not consistent with such models (7,8) but suggest instead that an appreciable fraction of the DNA is not on the outer surface.

MATERIAL AND METHODS

Nuclei were obtained by the method of Panyim *et al* (9), but Triton-X was omitted from the washing medium. Nuclei at a chromatin concentration of 5 mg/ml in 10 mM Tris pH 7.4, 1mM $CaCl_2$ were digested with 20 µg/ml of micrococcal nuclease (Worthington) for two minutes at 37°C. After stopping the digestion by the addition of EDTA to 2 mM and chilling on ice, the nuclei were lysed by a gentle stirring. The suspension was centrifuged at 2000 g for ten minutes and applied to a 5 m Biogel column. The central parts of the monomer fraction were concentrated by centrifugation and then ultrafiltration. Subunits were dialyzed against 10 mM Tris pH 7 , 0.5 mM EDTA in H_2O or D_2O for neutron scattering.

Subunits examined in the electron microscope by a freeze drying technique (10) or by freeze etching (4) had dimensions of 150 x 120 Å ± 15 Å. Very few aggregates or multimers were observed. Electron microscopy of spread subunit DNA after phenol extraction showed lengths equivalent to 185 ± 15 base pairs. Gel electrophoresis, kindly carried out in the laboratory of Dr.P.Chambon, showed a rather pure monomer band of 180 base pairs.

Subunits sedimented on sucrose gradients at 11S, and analytical ultracentrifugation showed that polydisperse material made up less than 10% of the samples. Gel electrophoresis of proteins from our chromatin and subunits (11) showed that all histones were intact. The non-histone protein (NHP)/DNA ratio for calf thymus subunits was 0.1 and for rat liver 0.2

Neutron scattering experiments were performed with the D11 diffractometer (12)of the Institut Laue-Langevin in Grenoble. For small angle scattering we used a detector sample distance of 5 meters with a wave length of 7 Å. No correction was found to be necessary for the $\Delta\lambda/\lambda$ spread of 8%. All curves were corrected for detector sensitivity and noise by referring to a long run on H_2O. At least 5000 chromatin counts were obtained at each radial position.

Guinier (14) found that the small angle scattering intensity (I) from all molecules at very small scattering angles obeyed :

$$\log I(h) = \text{constant} - \frac{h^2 Rg^2}{3}$$

$h = \frac{4\pi}{\lambda} \sin \theta/2$, where θ = the scattering angle and λ the wave length of the radiation; Rg, the globular radius of gyration for the scattering in H_2O and D_2O is given by: $Rg^2 = \Sigma a_i X_i^2 / \Sigma a_i$ where X_i = the distance of element i from center of gravity and a_i the scattering amplitude of this element.

If the average radial density of the elongated molecules

is approximated by a dense core and a less massive extended shell, two linear regions may be obtained in the customary plot. (13). If we consider the total amplitude scattered at the smallest angles, it is determined by R_g. But the amplitude scattered by the shell is always less than that of the core and is decreasing much more rapidly than that of the shell due to its larger dimensions. Accordingly, at larger angles, (or larger h) the overall scattering amplitude is mostly from the core.

The small angle scattering of a molecule in solution is a function of the sum of the scattering lengths per unit volume of the constituant atoms (ρ) minus that of the solvent. (ρ in H_2O ρ (H_2O) = -0.6×10^{10} cm^{-2} while in D_2O $\rho(D_2O) = 6.4 \times 10^{10} cm^{-2}$). If we break this sum over all constituent DNA atoms and protein atoms, we can obtain the relative contribution to the total scattering of protein to DNA at a given D_2O concentration. In H_2O this ratio equals .55 and equals 1.55 in D_2O (We note that for X-rays this ratio is .52 which is quite similar to that in H_2O with neutrons). In H_2O, taking the chemical composition into account, DNA contributes 60% of the total scattering, while for neutron scattering in D_2O, proteins contribute twice as much to the total as DNA. In 65% D_2O, the contribution of DNA to the total small angle scattering is effectively zero !

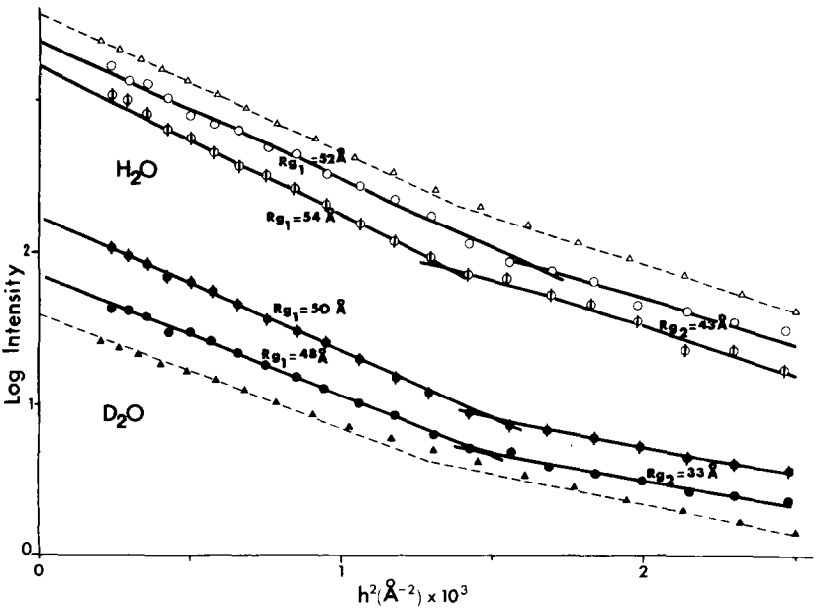

Figure 1. Experimental scattering curves from 5 mg/ml solutions of subunits from calf thymus in H_2O, (O) and D_2O (●), and from rat liver (ϕ) in H_2O and D_2O (●). Calculated scattering curves from double coil subunits with stoichimetry of calf thymus subunits are shown by (Δ) for H_2O and (▲) for D_2O contrasts.

RESULTS AND DISCUSSION

Guinier plots of some of our data are shown in Figure 1. As with the chromatin cross section X-ray (15) and neutron (4) scattering, two linear regions are present. We shall call the Rg derived from the first line Rg_1 and that from the second Rg_2. The presence and slopes of the two lines in both H_2O and D_2O infer the existence of a DNA-protein core and a DNA rich shell.

As shown in Figure 1, the Rg_1 of rat liver subunits is significantly larger than that from calf thymus. We attribute the larger Rg_1 of rat liver subunits to the presence of the added complement of NHP at the largest radii. Rg_2 is 20% larger in H_2O than in D_2O.This is an important feature of the scattering which must be explained by any chromatin model. Our preliminary results in 65% D_2O yield only one line with an Rg = 22 Å, from this Rg (protein), an Rg (DNA) of 60 ± 4 Å is calculated from the results in H_2O or D_2O.

It had been debated for some time as to whether most protein was on the inside or the outside of chromatin. Recently, a consensus has arisen favoring a central histone core. Although various models have been proposed, two will be considered here, as they lend themselves to direct comparison with our results. Baldwin *et al* (7) suggested a 60 Å diameter core of eight histones ($-H_1$) about which was wound two turns of a 55 Å pitch DNA coil. As they pointed out, the DNA structure in this model resembles that suggested by Bram and Ris (15) - a 45 Å pitch - 30 Å radius coil. To explain the neutron scattering from 140 base pair particles, Pardon *et al* (8) suggested models very similar to the model of Baldwin *et al* (7) in which the pitch is 45 Å. The Rg calculated for three subunit models are shown in table 1. The values for the protein core-DNA shell models are obviously too small to agree with the experimental Rg . Pardon *et al* (8) published an experimental Rg for the DNA alone, in the 140 base pair particle of 50.5 Å . Since the Rg of the 185 base pair particles are 52 Å and 54 Å in H_2O it is clear that the extra mass must lie at radii appreciably larger than 50.5 Å. In fact a Rg of 52 Å for a structure with a dense core suggests outer diameters between 120 and 150 Å

We should like to compare the experimental neutron

Table 1: Calculated globular radius of gyration (Å) for three models.

Model	H_2O	D_2O
Baldwin *et al.* (7)	48	40
Pardon *et al.* 8)	43	40
Double coil (4)	52	47
Experimental values	52	48

scattering to that expected from a model based upon electron microscopy of freeze fractured hydrated chromatin (4). Starting with a rough double coil model (4), by trial and error, we arrived at the model simulated in Fig.2. The electron micrographs suggested a model having two halves of a 200-250 Å pitch, 150 Å diameter coil joining the bottom of each subunit to the top of its neighbor. Upon entering the top of a subunit, the

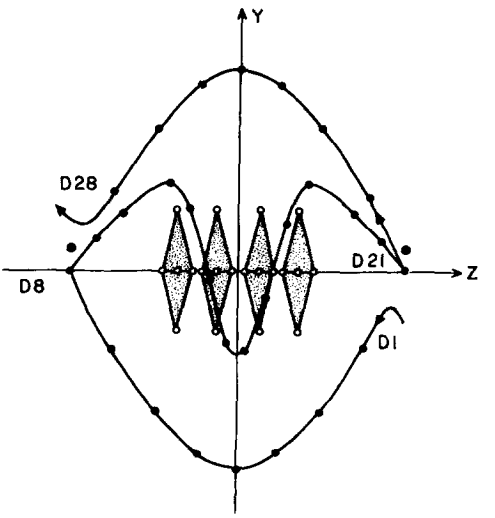

Figure 2. A sketch of a point simulation of a double coil subunit model. The 28 DNA points (●) which follow the numbered line are associated with two points (⊗) representing some NHP and are in a YZ projection. The arrows mark the entry and exit of DNA into the subunit. 20 histone points (O) are in four squares separated by 13 Å and are inclined slightly in the sketch.

DNA coiling direction changes sign and winds back with a pitch of about 45 Å and then changes its sense again before leaving the bottom of this subunit. The contour length of the two half outer coils per subunit in the electron micrographs is equivalent to 120 base pairs of DNA, leaving about 80 base pairs for the inner coil. The simplest core structure would be a 45 Å pitch, 30 Å radius coil, with about 20 base pairs joining the core and outer coil. (We do not believe that the DNA coiling is regular (15) and refined models may have to include sharp bends or folds).

Histones (excepting H_1) are assigned in this model to a central core. Elongated cylindrical core models gave a poor agreement to the data in D_2O. A globular structure for the histone core similar to that previously suggested (7) fits very well with the results. The model of Fig.2 is constructed out of points. However, scattering from any structure at a desired resolution is the same as that from sets of appropriately positioned points. This approximation will be valid as long as one is interested in the scattering curve at equivalent Bragg spacings which are large compared to the separation of the points in the model. In the case under study, where we are only interested in the radii of gyration region, a point model having merely the same radial distribution of scattering lengths as chromatin will have the same scattering.

In a solution where all orientations are equally probable, the scattering intensity can be calculated with the Debye equation

$$I(h) = \sum_{m,n} a_m \cdot a_n \frac{\sin hD_{mn}}{hD_{mn}}$$

where D_{mn} is the vector distance from atom or element m to n.

A calf thymus subunit with 185 base pairs and 120,000 Daltons of protein was represented by 22 protein and 28 DNA scattering points. To calculate the scattering in H_2O and D_2O, a (protein)/a(DNA) equalled .7 and 1.9 respectively. The coordinates and their a_m values were fed into a computer program previously used to calculate the scattering from the A and B forms of DNA. To better simulate a space filling model, we generally placed a 20 Å diameter sphere of uniform density at each structural element.

Fig.1 compares the calculated scattering for the double coil subunits of 185 base pairs to that observed for calf thymus subunits. The calculated curves agree with the data in both H_2O and D_2O. Rg_1 and Rg_2 are the same and the change in curvature is at the same values of h. Model calculations with two additional NHP points at 65 Å radii matched with the scattering from rat liver subunits.

Calculated scattering curves for other types of subunit models (7,8) or simple spheres or helices gave very poor fits to the experiments.

It is important to note that only models featuring about 40% of the DNA at radii of about 30-40 Å gave an agreement to the data in both H_2O and in D_2O. Moreover comparisons to the cross section scattering in H_2O, 65% D_2O and D_2O were also employed to refine the model. Consequently, as will be shown elsewhere, models of the kind shown in figure 2 are in agreement with the neutron scattering from intact chromatin.

The double coil model we have presented is not a unique solution to the data. Another model with the same radial distribution of mass with respect to both the center of gravity and the long fiber axis in chromatin would fit. However, these two conditions and the electron microscopy data are rather stringent. The model might also be consistent with many of the enzymatic digestion studies of chromatin which have found DNAse resistant particles with 80, 140, 185 and 200 base pairs (16, 2, 3, 1). We prefer to obtain higher resolution data and refine the model before making a detailed comparison to this literature.

ACKNOWLEDGEMENTS

We thank Dr.Ibel of the Institut Laue Langevin for his interest and invaluable assistance without which much of this work could not have been accomplished.

We are pleased to acknowledge financial support from the Centre National de la Recherche Scientifique and the Délégation Générale à la Recherche Scientifique et Technique and NATO.

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