Chromatin fiber dimensions and nucleosome orientation : a neutron scattering investigation

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ABSTRACT

Chromatin fibers were studied in solutions of mM monovalent salt by small angle neutron scattering. The variation of the cross section radius of gyration with H₂O/D₂O contrast shows that DNA is at much larger average radial distances from the fiber axis than histone. Consequently, the coils of DNA in a core particle must be approximately parallel to the fiber direction. The radii of gyration suggest that the maximum diameter of chromatin and nucleosomes is ~ 14 nm and that the DNA is distributed in two radial layers. The concentration dependence of the scattering maxima near 14 nm spacings furnishes independent support for a 14 nm external diameter and can be interpreted by a double DNA layer configuration.

INTRODUCTION

Chromatin is constituted of subunits called nucleosomes (1-6). The "canonical (7)" nucleosome can be subdivided into a dense core of histones and 140 base pairs of DNA called the core particle, and about 60 base pairs of DNA which link neighboring cores into a fiber. The chain of nucleosomes has been termed the unit fiber (8), and electron microscopy suggests nucleosome diameters ranging from 10 to 15 nm (3, 4, 6, 9, 10). Most electron microscopy and neutron scattering results are consistent with a loose super coil having a 25 - 30 nm external diameter with about 4 - 8 nucleosomes per turn (6, 10, 11; 12) in mM salt. At high concentrations in fibers, and in the presence of excess divalent ions, it has been suggested that the super structure is also a 30 nm diameter coil, but that the pitch is ca. 11 nm (13, 14, 15).

The general dimensions and overall protein-DNA distribution of core particles have been elucidated by neutron scattering (16, 17) and X-ray crystallography (18). However, less is known about the hydrated dimensions of nucleosomes and chromatin, and the nature of the linkage of nucleosomes into chromatin.

Small angle neutron and X-ray scattering can provide very useful infor-

mation on chromatin structure. At the very smallest angles so far studied - 150 to 45 nm equivalent Bragg spacings - the cross section radius of gyration (R_g) and mass per unit length (M/L) of the loose coil super structure were measured (19). It could be determined that the external diameter was 27 ± 3 nm and the mass compaction of the DNA in the super coiled fiber was 9.5. An earlier X-ray scattering study suggested a 5 nm cross section radius of gyration for a subunit in the unit fiber and a M/L of 22,000 Daltons/nm (20,10). A primary purpose of this report is to present the neutron scattering between 35 and 18 nm Bragg spacings, which is the region that depends on the radial; distribution of protein and DNA in a nucleosome cross section. Then, knowing the superstructure in 1 mM salt from other results (6,10,11,12,18, 21) we can demonstrate that reasonable values for the cross section radius of gyration of a nucleosome can be obtained. While our interpretation of the data may not be unique, it is supported by independent data.

The excess salt concentration has a very strong effect on chromatin structure. The early and important work of Zubay and Doty (22) showed that the intrinsic viscosity and light scattering do not vary in a plateau between .5 and 1.5 mM salt, but that at higher concentrations aggregation occurred, and below .5 mM salt, electrostatic repulsions become quite large and perhaps destroy the native structure. Similar transitions with salt have been observed and put into a more current context (23,24,25). Consequently, we have generally confined our scattering studies to the plateau region around 1 mM salt, but we note that our measurements at lower and higher ionic strength may suggest the onset of aggregation at about 10 mM salt and denaturation below .5 mM.

MATERIALS AND METHODS

Calf thymus chromatin was prepared by the method of Panyim et al. (26), but nuclei were dispersed in 1 mM NaCl, without shearing, using slow stirring. H_2O/D_2O exchanges were then accomplished by dialyzing for at least 20 hours against three changes of appropriate mixtures of H_2O and D_2O containing 1 mM NaCl. Electron micrographs of this chromatin showed contiguous, unaggregated linear arrays of subunits having a diameter of roughly 14 - 15 nm (10,11). DNase digest patterns of the chromatin obtained by Dr. N. Defer, were the same as those from intact nuclei. Chemical compositions were measured as previously described (20). DNA made up 44 % of the chromatin mass.

Neutron scattering experiments were performed with the D11 diffractometer (27) at the Institut Laue-Langevin in Grenoble. We employed a detector sample distance of 5 m with wavelengths between .5 and 1.0 nm and a $\Delta\lambda/\lambda$ of

8 %. Data were corrected for detector sensitivity by referring to a long run on H₂O and for noise by comparison to a run without neutrons. Measurements were made over a two year period on five different preparations.

The scattering from elongated molecules.

The small angle scattering from elongated molecules over a certain angular region is known to depend only upon the distribution of mass in a cross section (28). In this angular region of the scattering, ($hR \sim 1$), the intensity is given by :

$$h \times I(h) = M/L \times \text{constant} \times \exp(-h^2 R^2/2) \quad | 1 |$$

$h = \frac{4\pi}{\lambda} \sin \theta/2$ (the equivalent Bragg spacing = $2\pi/h$), where θ = the scattering angle and λ the wave length of radiation. M/L is the mass per unit length and R is the total cross section radius of gyration. R is defined by

$$R^2 = \sum a_i r_i^2 / \sum a_i \quad | 2 |$$

a_i is the scattering length of atom or structural element i , and r_i is its distance from the long axis of the particle. The ratio of a_i for histone protein averaged over all its atoms to that of a_i for DNA is .55 in H₂O and 1.5 in D₂O. Taking the chemical composition into account in H₂O, DNA contributes 60 % of the total scattering, but for neutron scattering in D₂O, proteins contribute twice as much to the total as DNA. In 65 % D₂O, only protein scatters as the small angle scattering of DNA is almost negligible.

If the scattering obeys equation |1| a plot of $\log[h \times I(h)]$ against h^2 will be a line. The slope of this line yields R , and from its intercept, $(h I)_0$, we calculate M/L (29,30). $M/L = \frac{(h I)_0}{tc N \bar{v}^2 (\rho_0 - \rho_{sol})^2 I_0} \quad | 3 |$

t is the sample thickness, c the concentration, N is Avogadro's number, $\bar{v}$ the partial volume; ρ_0 is the scattering density of the solvent corresponding to contrast matching and ρ_{sol} is the scattering density of H₂O. I_0 is proportional to the neutron flux and can be derived from the H₂O inelastic scattering (31). Alternatively, M/L can be obtained by scaling the neutron scattering, curves with X-ray scattering data. Since at equivalent contrasts ($\sim 10\%$ D₂O) both exhibit a similar R_1 . Then matching the two curves in the R_1 region scales the intercepts and gives a M/L of 22,000 Daltons/nm. We have presented elsewhere (11) the scattering calculated from a straight chain of subunits each having a single DNA shell about a protein core.

This is the single DNA shell model which best fits our data between 32 and 20 nm equivalent Bragg spacings. Plotted in the customary manner as $\log(h \times I(h))$ vs h^2 the scattering is linear to values of hR , ≈ 2.5 . The slope

gave a graphical cross section radius of gyration which equalled that calculated with equation |2| within $\sim 5\%$.

Scattering from loose coils.

For a straight chain of subunits, equation |1| will hold at the smallest angles and the slope will yield the radius of gyration of scattering density projected onto a cross section perpendicular to the fiber direction. If the chain of subunits is slightly bent or coiled, equation 1 will still be valid over a certain angular range and the effect of coiling will be an increase in slope of the customary plots at the smallest angles. At the other extreme, a very tightly coiled chain will scatter like a hollow cylinder, and the cross section scattering from subunits cannot be extracted from the total. In order to determine the range of validity of equation |1| and the effect of coiling on R_1 obtained from the graphs, we have calculated with the Debye equation(32) the scattering from a great number of different structures.

As mentioned above, nearly all classical and freeze fracture electron microscopy studies of chromatin in the mM salt employed here would be consistent with a loosely coiled chain of subunits (20, 6, 10, 12). A super coil having an outer diameter of ~ 27 nm, pitch of 30 - 45 nm and an axial translation of about 7 nm would be in general agreement with the electron microscopy, and very small angle neutron scattering (19) results. This super coil is only compacted 1.4 X that of a straight chain. In figure 1, we present a calculated scattering curve for such a super structure of 12 nm diameter spheres. At very small angles the $\log(h \times I(h))$ vs h^2 plot is linear out to about 5 nm spacings with a slope corresponding to $R_0 = 8.0$ nm which is the cross section radius of gyration for the super structure of these spheres. At an h corresponding to about 40 nm there is an abrupt inflection in curvature and then the intensity falls off following another line to about $h \times R_1 \sim 3$. (We note that the equivalent Bragg spacing of this inflection is a function of the superhelical pitch and radius.) The slope of the second line yields a graphical R_1 , which we call R_1 , equal to 4.1 nm. The expected cross section radius of gyration of a 12 nm sphere is 3.8 nm ($r \times \sqrt{2/5}$). From the intercept of the second line, we calculate the M/L and this value equals that expected from a straight chain of spheres to within about 5 %. Similar calculations with different sizes, shapes and density distributions show that the graphical R_1 and M/L equal the known values when the super helical compaction is less than two fold. (Sphere diameters between 7 and 14 nm, and protein cores with two DNA shells (11,19) were some of the examples calculated.) On the other hand,

when the compaction is greater than two, extraction of the cross section parameters is quite difficult.

Justification for our cross section scattering treatment.

Chromatin exhibits similar scattering curves to those for loosely coiled subunit models out to about 18 nm, and it is reasonable that we treat our data in the same manner. However, justification for any scattering treatment can only be provided by comparison to independent evidence.

An absolute intensity determination of M or M/L from the intercept of the customary lines is generally taken as good evidence that the data has been correctly interpreted. The M/L we obtain (see below) is 23,000 Daltons/nm which translates to a DNA compaction of 5.5. This value is in accord with the electron microscopy result of 6 ± 1 (6,33) of the literature.

The external diameter of 13 - 14 nm obtained from the cross section radii of gyration equals that measured in our freeze fracture electron micrographs (10,11) and also that determined from the nucleosome neutron scattering (34). R_1 varies with solvent contrast in an analogous manner to that of the isolated-nucleosome scattering (see fig. 4). Finally, the R_1 values of a series of chromatin having different NHP contents, vary as expected from the chemical composition and an NHP location external to the histone core (11).

One must be careful not to allow a conformity to current ideas to bias treatment of the data by selection of curves or by choosing convenient parameters. Evidence that this is not the case for this work is that our R_1 and M/L equal those obtained from X-ray studies in 1968 (20,10).

Since aggregation is the bane of scattering experiments, it is prudent to consider arguments in addition to those above which are specifically pertinent. Electron microscopy by freeze-etching of these identical preparations in mM salt show no indication of significant aggregation (10,19). Aggregation should be appreciably reduced by nuclease fragmentation, H1 extraction or 650 mM salt, but after such treatments chromatin exhibits customary plots consistent with our interpretations (11,14). Likewise, lowering the concentrations should reduce interparticle interactions but R_1 increases. Moreover, an R_1 due to aggregation or some other higher order arrangements should not change much with solvent contrast, yet, as seen in figure 4 R_1 falls by more than 30 % when DNA is contrast matched. Interparticle interactions do make significant contributions to the scattering at higher concentrations than used for our R_1 and M/L results, and at salt concentrations above 10 mM.

RESULTS AND DISCUSSIONVery small angle scattering maxima.

The early scattering patterns on undispersed chromatin solutions showed a maximum at about 40 nm (35). This maximum was taken as the first evidence that the ~ 10 nm unit fiber of nucleosomes had a regular higher order structure in solution (35). It was then suggested that this peak arose mostly from the first order Bessel function of a super helix. Now, after more extensive studies, we have found that this maxima is replaced by an inflection in curvature for nuclease digestion fragments (19), after dispersal at concentrations below about 6 mg/ml or in the presence of 10 mM salt. On the other hand, the inflection in curvature is very similar to that calculated for the super structure derived from electron microscopy or very small angle scattering (19). (See also figure 1). Consequently, it is now more reasonable to suppose that the very pronounced 40 nm maxima at higher concentrations have a large contribution from vectors perpendicular to the fiber.

Treatment of small angle scattering in 1 mM NaCl.

Figure 1b and c present typical plots of $\log(hxI(h))$ vs h^2 for calf thymus chromatin. Nuclease digestion fragments 150 nm in length exhibited the same scattering as intact chromatin (11,19). A linear region between $.25 < h^2 < .8 \times 10^{-3} (\text{\AA}^{-2})$ is observed as with the loose super helical models (see Fig. 1a, 1b and 1c). In this report we deal only with this R_1 region which the model calculations (Figure 1a and ref.11) and M/L show to be functions of the radial distribution of mass in a nucleosome. That the data is in fact, linear in this region provides some support for our interpretation. At smaller h the scattering rises above the R_1 line. This inner region of the scattering was dealt with in a previous publication and shown to be entirely compatible with our loose super coil model (19). We note that experimental problems prevent a quantitative comparison of the data in the inner regions to the model curve. The first three points are from detector elements separated by only 5, 6 and 7 cm from the central beam stop and their contributions is often neglected due to instrumental parasitic scattering and smearing effects. This region is studied by employing a two fold larger sample detector distance or wave lengths, as in ref. (19). The model curve in Figure 1a is for infinite dilution and perfect collimation. At h^2 larger than $.008 (\text{\AA}^{-2})$ both the data and model curves are no longer linear in the customary plot. Lines were drawn in the R_1 region in two manners : 1) By eye, three lines were drawn to determine the minimum, maximum and average R_1 within the

Fig. 1: a) Theoretical scattering from the loose 42nm pitch, 7nm radius super coil of uniform spheres described in the text R_1 is extracted from the graph line between $.25 < h^2 < .8 (\text{\AA})^{-2} \times 10^3$.

b) Experimental neutron scattering from one chromatin preparation at 4.5mg/ml, 1mM NaCl in various D_2O/H_2O mixtures. The graphical cross section radius of gyration, R_1 , is proportional to the square of the slope of the line drawn by eye in the same region of h for all experimental and model curves. The three inner most points, corresponding to radii of 5.6 and 7cm, are imprecise are usually neglected.

experimental error. (The lines in Figure 1b were so drawn.) 2) Least square fitting by a computer program (Univac, 1110 Library) was used as in Figure 1c. Comparing the R_1 values for data treated in manners 1 and 2 (Fig. 1b and c) shows that they both give the same results within the experimental error. Comparison of R_1 values at different concentrations and on three different preparations is presented in Figure 3. It can be seen that R_1 is reproducibly obtained and that the variation with concentration is less than the experimental error of $\pm 5\%$.

As previously mentioned our model curves and M/L suggest that R_1 equals the actual cross section radius of gyration of a nucleosome to within 5%. However, we cannot be certain that the error is not two, or three fold larger. Nevertheless, qualitatively, it can be concluded that the slope of

Fig.1c: Least square fitting by computer to the scattering from a 6 mg/ml calf thymus chromatin solution in the R_1 region. The line $y = Ax + B$ (where B represents $\text{Log}_e (h I_0)$, and a equals $(R_1)^2$) is drawn for each contrast.

the scattering curve in a region which does depend on the radial distribution of mass in a cross section decreases significantly as DNA is contrast matched. Consequently, our major conclusion from Figure 4 (see below) would remain unaltered.

Mass per unit length of unit fibers.

Intercept values of the lines between 30 and 12 nm were extrapolated to zero concentration. The results for five $\text{H}_2\text{O}-\text{D}_2\text{O}$ contrasts are plotted in figure 2. In figure 2 the plot is linear and passes through zero contrast at 50.5 % D_2O .

The line in figure 2, and the partial specific volume of calf thymus chromatin (22), incoherent scattering, and loss factor (41) were used to calculate the mass per unit length. We obtain a M/L of $23,000 \pm 2,000$ Daltons/nm. This M/L is only slightly larger than reported from the X-ray scattering using the R_1 intercept - 22,000 (20,10). Our M/L on calf thymus chromatin, at a first glance, resembles a M/L published for rat liver chromatin (36). In that study (36), however, the 30 - 35% mass contribution of NHP in rat liver chromatin was neglected, and, if taken into account, would give an M/L of about 20,000 Daltons/nm which agrees reasonably well with our result.

In the present paper, we remind the reader that we use the M/L for justification of our data analysis and feel that the X-ray values (20) are more reliable. Still our neutron scattering provides some support for our previous X-ray scattering value.

Fig. 2 : Dependence on solvent contrast (ρ_{sol}) of the zero angle intercept of the $\log_e |hI(h)|$ plots.

Cross section radii of gyration and the external diameter.

R_1 values after extrapolation to zero concentration are plotted in figure 4 as R_1^2 against $1/\bar{\rho}$ ($\bar{\rho}$ is the average of the solute contrast at various H_2O/D_2O mixtures) following the suggestion of Stuhrmann (37). When $1/\bar{\rho} = 0$, the solute contrast is infinitely large and all compounds scatter with uniform density. The data is linear within the experimental error and passes through the infinite contrast point when $R_1 = R_{1C} = 4.65$ nm. R_{1C} is the cross section radius of gyration of the solvent-excluding solid. The smallest external diameter consistent with R_{1C} is 13 nm, which is that of a uniformly filled cylindrical cross section ($13.1 = 2 \times \sqrt{2} \times 4.65$).

It is instructive to compare the cross section area of such a uniform solid cylinder 13.1 nm in diameter to the actual "dry" cross section area calculated from the experimental M/L and $\bar{v}$: $A(R_{1C}) = (13.1/2)^2 \pi = 132$ nm². The slope of the line in Fig. 2 yields A which = 110 nm². The similarity of the two values of A is further independent support for our treatment.

$$\text{But, } A(M/L) = \frac{\bar{v} \times 10^{21} \text{ nm}^3/\text{cm}^3 \times M/L}{6.02 \times 10^{23}} = 26 \text{ nm}^2$$

The ratio of the area from the R_{1C} to that from the M/L (f) is ~ 5 , and as pointed out by Kratky (29) it is a measure both of the deviation from compact cylindrical shape and of internal hydration. Consequently, we can conclude that chromatin has a very loosely packed hydrated structure. Moreover, returning to the derivation of the external diameter from R_1 , for an extensively hydrated or asymmetric cross section the external diameter must be appreciably larger than 13 nm - at least 14 nm. Quantitative measurements of electron micrographs of hydrated chromatin replicas also give an external diameter of about 14 nm (11).

Orientation of nucleosomes in chromatin fibers.

Nucleosomes in chromatin can be ordered in one of two general manners :
 1) histone cores could stack top toward bottom of one another with the axis of the DNA core particle coil approximately parallel to the fiber direction, or
 2) nucleosomes could be arranged side by side so that the axis of the core particle coils was approximately perpendicular to the vector between neighbors. For the former case, DNA does not touch the fiber axis and a projection onto a cross section would exhibit a histone core, with a DNA shell at larger radii. In case 2, the DNA intersects the fiber axis and in a cross section projection there would be little or no difference between the DNA and protein radial distributions. The R_1 of protein is

estimated by extrapolation of the line in figure 4 to $\bar{\rho}$ equivalent to 65 % D₂O, and R_1 of the DNA is obtained at $\bar{\rho}$ equivalent to 40 % D₂O. The cross section radius of gyration of the protein is $3.3 \pm .2$ nm while that of the DNA is $5.5 \pm .2$ nm. Clearly, these results are only compatible with a cross section having a central protein core and a shell of DNA. Then the orientation of the histone cores and DNA coiling in nucleosomes is approximately parallel to that of the fiber direction (case 1). (Fig. 3 of ref.19 shows one general nucleosome orientation which would be in agreement with the cross section radii of gyration.)

The results can be described in a more quantitative fashion (37) by referring to the slope (α) of the R_1^2 vs $1/\bar{\rho}$ curve (fig. 4) near $1/\bar{\rho} = 0$. For our calf thymus chromatin $\alpha = +8.5 \times 10^{-4}$. If a line were drawn through the points in fig. 4 for calf thymus nucleosomes near $1/\bar{\rho} = 0$ the slope would be very similar to that of intact chromatin. This suggests that the asymmetric histone core-DNA shell distribution in nucleosomes is similar to that in a cross section of chromatin.

To a first approximation, the R_1^2 vs $1/\bar{\rho}$ plot is linear, which implies that the center of mass of protein and DNA in chromatin are not far apart (37). Although our errors compare to those in other studies (i.e. (16)), the reader will note that by taking the extremes of the error bars in figure 4 other lines or even a parabola can be readily drawn through the data. Consequently, quantitative calculation of α for various models would not seem to be justified.

DNA is distributed in two radial layers and a consideration of models.

Neutron scattering (17,42) and crystallography (18) show that core particles are 5.7 nm high and that the axis of the DNA core particle coiling or folding is parallel to the small dimension. From our results it follows that the 5.7 nm height of a core particle must be approximately parallel to the fiber direction. The globular radius of gyration of the core particle DNA is given by : $R_g^2(\text{DNA}) = R_1^2(\text{core particle DNA}) + (\text{height})^2/12 = (5.05 \text{ nm})^2$ (16). Then R_1 for the core particle DNA = 4.8 nm. For chromatin the total R_1 of the 196 base pairs in a subunit is : from equation | 2 | the square of the contribution from the core particle plus that in the linker :

$$R_1^2(\text{DNA}) = \frac{140}{196} (4.80^2) + \frac{56}{196} \cdot R_1^2(\text{linker}) = (5.5 \text{ nm})^2$$

R_1 of the linker is then 6.8 nm. This would imply an external diameter for a peripheral shell of about 15 nm which is what we observe in our electron micrographs under these conditions (10,11).

Figure 3: The variation of graphical R_1 with concentration for several different runs. ● R_1 from lines drawn by eye as in Figure 1b; ○ from least square computer fits as in figure 1c.

Fig. 4 : Contrast variation of R_1 for calf thymus chromatin after extrapolation to zero concentration. N denotes the globular radii of gyration of calf thymus nucleosomes in D_2O and H_2O from ref. (34).

The models of Pardon et al. (17) and Finch et al. (18), each provides satisfactory but not unique interpretations for the neutron and X-ray data, yet they do not tell us about the structure of the 50 - 60 base pair linker. These models could be joined by a linker coil of 6-7 nm center radius and then they would explain our cross section scattering results on chromatin.

An alternative model has been suggested in which a core particle contains about 70 b.p. in a ~ 3 nm radius coil, and the remaining DNA is in two 6.5 nm radius coils which wind in opposite handedness to the central coil (10,34,38). In chromatin the extra 50 - 60 base pairs would join to the peripheral coils and indirectly link adjacent core particles bottom to top of neighbor. Crick (39) has demonstrated that an indirect linkage by a DNA exterior to a central DNA - protein core is consistent with what is known about chromatin topology. It will be shown elsewhere that a double coil model is in agreement with all the neutron scattering on core particles, nucleosomes, and chromatin; but scattering data alone cannot prove a given model. Nevertheless, it is pointed out that this double coil model suggests a direct interpretation for the observed probabilities of all the DNase I cuts on core particles (38).

Analysis of our previous nucleosome scattering results.

We have recently placed our previously published (34) 185 base pair nucleosome scattering data on an absolute scale by comparison to the H_2O incoherent scattering. The molecular weight obtained for calf thymus nucleosomes is $290,000 \pm 3,000$ Daltons. (Rat liver nucleosomes which contained about 20 % NHP by weight had a molecular weight of $\sim 300,000$ Daltons) Our calf thymus nucleosomes contained about 10 % histone H1 and NHP. The measured molecular weight agrees reasonably well with the 260,000 Daltons expected.

In the presence of NHP and H1 one expects 185 base pair particles upon short digestion (40,7). However, nucleosomes used in another study contained no H1 nor NHP (41) and their DNA content was recently reported to be between 155 and 200 b.p. (42). Our globular radius of gyration is about 5 nm, as is that reported by Harrison and Kornberg (43), while for the other study (41) an appreciably smaller value (4.3 nm) was reported. The smaller R_g could well reflect a smaller particle. The agreement of the molecular weight with expectations, and the fact that R_g is similar to another study leads us to present the H_2O and D_2O data on an R_g^2 vs $1/\bar{\rho}$ plot (figure 4). Evidently, it would be imprudent to extrapolate to low contrasts with only H_2O and D_2O points. Nevertheless, two important conclusions can be made :

1) The slope of the plot at high contrasts is similar to that of intact chromatin cross section scattering, 2) The value of R_{gv} obtained at $1/\bar{\rho} = 0$ is $5.0 \pm .2$ nm. From R_{gv} the minimum outer diameter is obtained by assuming a spherical uniform cross section which is 12.9 nm ($2 \times \sqrt{5/3} \times 5.0$). This gives an independent corroboration for the cross section scattering results. In a similar manner we compute the volume of a nucleosome from the molecular weight and $\bar{v}$, and that from a uniform 12.9 nm diameter sphere.

$$v(R_{gv}) = \frac{4}{3} \pi (6.5)^3 = 1150 \text{ nm}^3$$

$$v(M) = \frac{290,000 \bar{v}}{6 \times 10^{23}} \times 10^{21} \text{ nm}^3/\text{cm}^3 = 327 \text{ nm}^3; \text{ then } f = 3.5.$$

We can now estimate the dimensions of a nucleosome. Two dimensions must be in excess of 13 nm - at least 14 nm, while the height of nucleosomes calculated from M/L in chromatin suggests that the orthogonal extent be on the order of 10 to 12 nm.

Concentration dependence of the ~ 11 nm maximum suggests a 14 nm dimension.

Our scattering results from dilute solutions require an external diameter of 14-15 nm. We suggest that the data at ten fold higher concentration can also be interpreted by a ~ 14 nm diameter. Traditionally, chromatin has been studied with X-rays in fibers at 98 % relative humidity which corresponds to a chromatin concentration of approximately 45 %. A scattering maximum is observed near 11 nm at this concentration (44,45,46) and it has long been known that it shifts toward 8 nm upon further drying (23,34 and table 1).

Table 1

Equivalent Bragg spacing of scattering maxima at different chromatin concentrations

Chromatin concentration (per cent weight)	Spacing (nm)		Reference
	first maximum	second maximum	
10	13 - 14	7.5	figure 5
20	13 - 14	7.5	(46)
20	13 - 14(a)	7.	(47)
35	12	-	(46)
45	11	5.5	(44,45,46)
80	8	3.7	(44,45)

(a) Taken from position of maximum inflection in published scattering curve.

More recently, it was found that this spacing shifts toward 13 to 14 nm as the concentration is lowered near 20 % (46). Sperling and Klug (47) also presented a scattering curve with a strong shoulder near 14 nm for a 20 % gel. These authors suggest that the lower the concentration required for obtaining a ca.11 nm maximum, the more native is the chromatin. Our chromatin exhibits a strong maximum near 13.5 nm at a concentration of only 10 %, suggesting by this criteria that it is quite native. The scattering maxima, given in fig. 5, are sharper than in previous studies. We partially attribute this to the five to ten fold better resolution of the diffractometer-monochromator employed (48) compared to that used in other work. In figure 5 the second maximum is seen near 7.5 nm. Maxima at ca.7.5 nm have been previously reported for 20 % gels of chromatin (46) and at 6.3 nm for intact nuclei (38). The original interpretation (and that which we believe to be the best) for the origin of the ca.11 nm spacing was side by side packing of chromatin units having a dimension similar to this spacing (44). Alternatively Carlson and Olins (13) suggested that this maximum results from the pitch of a tight super coil and that the pitch is due to the effective diameter of a nucleosome. In either case the simplest explanation for the decrease in spacing from 14 to 8 nm is a parallel reduction in the effective separation between fibers or nucleosome

Fig. 5 : Neutron scattering from a 10 % calf thymus chromatin gel (26) in 1 mM NaCl - H₂O with a high resolution diffractometer and graphite monochromator (48). The sample was kept at 4° C and scanned with a step counter for 30 hours. Background and solvent were subtracted from the data using separate runs, scattering maxima are observed at roughly 13.5, 7.5 and 5.5 nm.

dimensions with increasing concentration of chromatin.

The concentration dependance of these spacings can be interpreted by our double DNA coil model, as follows : at concentrations of 10 - 25 %, neighboring fibers or nucleosomes begin to pack with a separation equal to that of their external diameter - 14 nm in the model. As the concentration increases, the outer coils, which occupy only a small part of the peripheral volume, interpenetrate with their neighbors and come in contact with their neighbors core. The separation is then $(14 + 8)/2$ or 11 nm, which corresponds to the spacing observed at concentrations between 35 and 45 %. At even higher concentrations, the 8 nm central cores pack with the smallest possible separation. We note that salt and divalent ions will neutralize the highly charged chromatin and will certainly affect the variation of these spacings with concentration.

Considerations of the ion concentration in vivo and in vitro.

It has been shown in several studies that the ion concentration in the chromatin environment plays a very important structural and functional role (23,24,25). Although one often assumes that the monovalent ion concentration is .15 M and divalent concentration 1 - 10 mM in the nucleus, the actual free ion concentrations may be rather different.

The ion contribution of chromatin is often neglected. Chromatin is a very highly charged poly-ion in which as many as 50 % of the DNA phosphate groups may be free (50,51), and therefore carry along free positive counterions. Negative charges on the proteins also make a significant contribution. Taking 50 % of the DNA mole phosphates for the free positive ion contribution, a 1 % solution would contain at least 7 mM ions and a 30 % gel 0.2 M. The total chromatin concentration, and ion concentrations in nuclei have been reported to be 30 % (49), and .4 M (52). Locally, the ion concentration could exceed .6 M.

For divalent ions the situation is the inverse. We know approximately the total or bound ion concentration (53) : $\text{Ca}^{++}/\text{PO}_4 = .1$, but do not know the free divalent ion concentration. The free Ca^{++} concentration has been well measured in the cytosol to be $2 \times 10^{-6} \text{ M}$ (54). Since the nucleus and cytosol are in equilibrium through the 80 nm nuclear pores, the free ion concentration ought to be only a few μM . Clearly, the ion concentrations in vivo and their effects on chromatin structure require further study.

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