Higher Order Folding of Two Different Classes of Chromatin Isolated from Chicken Erythrocyte Nuclei. A Light Scattering Study†

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ABSTRACT: Bulk chromatin fragments were excised from chicken erythrocyte nuclei by digestion with micrococcal nuclease. Fractionation into S chromatin (soluble at physiological ionic strengths) and I chromatin (insoluble at physiological ionic strengths) was achieved by dialysis against buffers containing 0.15 M NaCl. The effects of NaCl concentration on the molecular dimensions of S and I chromatins were determined by dynamic and static light scattering. Series of fragment lengths were obtained by gel filtration of S and I chromatins under ionic conditions which lead to maximal intramolecular compaction. Hydrodynamic radii and radii of gyration were determined for fragment lengths ranging from 8 to 53 nucleosomes. These data are in excellent agreement with calculations for extended helical structures. Close-packed solenoidal or superbead structures are not compatible with these data. Comparisons of molecular dimensions derived from light scattering and electron microscopy indicate that considerable shrinkage of chromatin fragments can occur when common methods of sample preparation are used for microscopy.

DNA of eukaryotes is organized into chromatin subunits known as nucleosomes. A nucleosome contains approximately 200 base pairs of DNA which are wrapped around the outside of an equal mass of globular core histone proteins (H3, H4, H2A, and H2B). Approximately one molecule of lysine-rich histone (H1 and H5) is bound to each nucleosome. A linear array of repeating nucleosomal subunits forms the basis for higher order folding of chromatin in the nucleus. Although significant progress has been made in understanding mononucleosome structure, relatively little is known about the details of higher order folding (Kornberg, 1977; Felsenfeld, 1978; McGhee & Felsenfeld, 1980).

Numerous levels of folding chromatin chains which lead up to the most extreme level of compaction found in the metaphase chromosome have been reviewed by Ris & Korenberg (1979). Most of the recent attention has been focused on understanding the details of nucleosome packing in chromatin fibers which display 250–300-Å diameters in classical electron microscopic studies. Finch & Klug (1976) proposed that this structure is achieved by continuous helical coiling of a chain of contiguous nucleosomes into a close-packed helix or "solenoid" of diameter ~300 Å. This model has been supported by recent electron microscopic studies on isolated chromatin (Thoma et al., 1979)) as well as light scattering (Campbell et al., 1978) and neutron scattering (Suau et al., 1979) studies on chromatin fragments in solution. Electrooptical studies of cross-linked chromatin, conducted at low ionic strengths in the presence of Mg^{2+}, also favor the solenoidal model (McGhee et al., 1980a,b; Lee et al., 1981). An alternative model for the 250–300-Å fiber is based on the discontinuous packing of compact globular clusters of 6–14 nucleosomes known as superbeads (Hozier et al., 1977; Renz, 1979; Renz et al., 1977; Stratling et al., 1978; Itkes et al., 1980; Jorcano et al., 1980; Stratling & Klingholz, 1981). These discrete superbead structures are thought to give rise to the "knobby" appearance of 250–300-Å fibers.

Several apparently conflicting reports of chromatin solubility in aqueous solutions at physiological ionic strength prompted a detailed examination of the release of bulk chromatin fragments from chicken erythrocyte nuclei by digestion with micrococcal nuclease (Fulmer & Bloomfield, 1981). These studies indicated that bulk chromatin preparations contain two different classes of chromatin. Approximately 40% of the total chicken erythrocyte genome (S chromatin) was soluble at physiological ionic strengths while the remaining ~60% (I chromatin) was insoluble above 0.1 M NaCl. Kinetic studies indicated that these two classes of chromatin are different in situ and are not related in a precursor–product fashion resulting from nucleolysis. The present study reports effects of NaCl concentration on higher order folding of both S and I chromatins in dilute aqueous solutions devoid of divalent cations. Ionic conditions which impose maximal compaction of these two different classes of chromatin were determined. Series of fragment lengths ($8 \leq \bar{N} \leq 50$) were obtained under these ionic conditions by gel filtration chromatography. Molecular dimensions were characterized by translational diffusion coefficients measured by quasi-elastic light scattering (QLS)[1] and by radii of gyration measured by classical light scattering. These data were then interpreted in terms of various possible modes of higher order folding of polynucleosome chains. Current models are compared to the results of this study.

Materials and Methods

Sample Preparation. Soluble chromatin fragments were isolated from chicken erythrocyte nuclei by digestion with micrococcal nuclease. Fractionation of bulk chromatin into S chromatin (soluble at physiological ionic strengths) and I chromatin (insoluble at physiological ionic strengths) was achieved by dialysis against 0.15 M NaCl, 10 mM Tris-HCl, and 1 mM EDTA, pH 7.5. Purified S and I chromatins were then fractionated according to size by gel filtration chromatography on Bio-Gel A-150m. Columns were equilibrated at

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[1] Abbreviations: Tris, tris(hydroxymethyl)aminomethane; EDTA, ethylenediaminetetraacetic acid; $NaDodSO_4$, sodium dodecyl sulfate; QLS, quasi-elastic light scattering; D, translational diffusion coefficient; R_H, effective hydrodynamic radius; R_G, radius of gyration; $\bar{N}$, weight-average number of nucleosomes per chromatin fragment.

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4 °C with 0.15 and 0.08 M NaCl containing 10 mM Tris-HCl and 1 mM EDTA, pH 7.5, for fractionation of S and I chromatins, respectively. $NaDodSO_4$–polyacrylamide gel electrophoresis of S and I chromatins revealed no detectable proteolysis of histone proteins within 10–14 days after isolation of nuclei when samples were stored at 4 °C. See Fulmer & Bloomfield (1981) for experimental details.

Undiluted and diluted (2–4-fold, with column fractions eluting prior to the void volume) chromatin fractions from the A-150m elution profiles were prepared for light scattering studies by centrifugation at 500*g* for 6–18 h at 4 °C in 8 × 175 mm Pyrex culture tubes. These tubes were transferred directly from the centrifuge to the light scattering apparatus for data acquisition. This method of sample clarification resulted in exceptionally clean samples. The intensity of light scattered from these samples remained constant (±2–5%) for at least 1 h after removal from the centrifuge. Absorbances at 258 nm were determined for aliquots of various samples which were removed from the vicinity of the scattering volume both before and after centrifugation. These measurements indicated that no detectable solute redistribution had occurred upon centrifugation.

Light Scattering Apparatus. Both quasi-elastic and total intensity light scattering measurements were made with an instrument which has been described previously (Pletcher et al., 1980). All data were recorded at 20 °C at a laser wavelength of 5145 Å.

Quasi-Elastic Light Scattering (QLS). The translational diffusion coefficient, D, was determined by QLS measurements [see reviews by Schurr (1977) and Bloomfield & Lim (1978)]. The normalized homodyne autocorrelation function $C(\tau)$ of the scattered light intensity may be written as

$$C(\tau) = e^{-2\Gamma\tau}$$

where $\Gamma = q^2D$ and q is the scattering vector. The logarithm of the experimentally determined autocorrelation function was fitted to a quadratic function

$$\tfrac{1}{2} \ln C(\tau) = A - \Gamma\tau + \sigma^2\tau^2/2$$

according to Koppel's (1972) cumulant method. The coefficient σ^2 is primarily a measure of sample polydispersity. The ratio σ^2/Γ^2 was found to be around 0.03 and independent of q^2 for all chromatin samples reported in this study. This value is close to a practical minimum for QLS studies of biological macromolecules and indicates a rather narrow size distribution of fractionated chromatin samples. The effective hydrodynamic radius R_H was calculated by the Stokes–Einstein relationship

$$R_H = kT/(6\pi\eta D)$$

where η is the viscosity of the solution.

Total Intensity Light Scattering. The weight-average molecular weight, M, and radius of gyration, R_G, of fractionated chromatin samples were determined from the excess light scattering intensities by the following relationship (Tanford, 1961):

$$\frac{B(dn/dc)^2c}{I - I_B} = \frac{1}{M_{app}} = \frac{Kc}{R}$$

where I and I_B represent the scattering intensities of sample and solvent, respectively. M_{app} is the apparent molecular weight, uncorrected for concentration- or angle-dependent effects, K is an optical constant, and R is the Raleigh ratio. The concentration of chromatin (c) was expressed in milligrams per milliliter and determined by the absorption at 258 nm by using an extinction coefficient of 10 cm^{-1} $(mg/mL)^{-1}$. The proportionality constant B was determined as described by Pletcher et al. (1980). Refractive index increments, dn/dc, were determined as described below. Excess light scattering was collected over a range of scattering angles from 30 to 130° and chromatin concentrations from 0.01 to 0.28 mg/mL. These data were extrapolated to $\theta = 0°$ and $C = 0$ by the usual Zimm plot method, Kc/R vs. $\sin^2(\theta/2) + 10c$ [see Tanford (1961)], to yield M and R_G. Molecular weights were converted to nucleosome equivalents, $\bar{N}$, by using a mononucleosome molecular weight of 2.7×10^5.

Refractive Index. Refractive index measurements were made at a wavelength of 5460 Å on a model B-S differential refractometer (Phoenix Precision Instruments Co., Philadelphia, PA) at 20 °C. The values of dn/dc for both S chromatin in 0.15 M NaCl, 10 mM Tris-HCl, and 1 mM EDTA, pH 7.5, and I chromatin in 0.08 M NaCl, 10 mM Tris-HCl, and 1 mM EDTA, pH 7.5, were 0.184 mL/g.

Measurement of Sedimentation Coefficients. Sedimentation coefficients were measured by using a Beckman Model E ultracentrifuge equipped with ultraviolet absorption optics. The An-J rotor was operated at 16 000 rpm at 21 °C. The optical patterns were recorded on Kodak film and scanned with a Quick Scan densitometer (Helena Laboratories). Sedimentation coefficients were calculated from the mid position of the boundaries and corrected to standard conditions ($s_{20,w}$) in the usual manner (Svedberg & Pederson, 1940).

Electron Microscopy. S chromatin in buffer containing 0.15 M NaCl and I chromatin in 0.08 M NaCl were fixed at 0 °C with 0.1% glutaraldehyde for ~16 h. Samples at ~0.05 mg/mL were adsorbed to carbon-coated grids rendered hydrophilic by glow discharge and negatively stained with 2% uranyl acetate. Specimens were examined with a Hitachi HU-11C electron microscope at an acceleration voltage of 75 kV at calibrated primary magnifications of 50 000–60 000×.

Results

Preparation and biochemical characterization of chromatin samples used in this study were described by Fulmer & Bloomfield (1981). Both S (soluble at physiological ionic strengths) and I (insoluble at physiological ionic strengths) chromatins contained a full complement of core and lysine-rich histones when compared to whole nuclei by $NaDodSO_4$–polyacrylamide gel electrophoresis. These two classes of chromatin contained 1.00 ± 0.05 g of total protein per g of DNA. Nonhistone proteins were absent from both of these preparations as judged by $NaDodSO_4$–polyacrylamide gel electrophoresis. This section will begin with examples of our physical data before presenting the results of this study. Finally, details of our hydrodynamic modeling are described.

A typical autocorrelation function, $C(\tau)$, is shown for S chromatin in Figure 1A. The upper data in Figure 1A correspond to the logarithmic ordinate on the right. Minimal deviations from linearity indicate that the relaxation of $C(\tau)$ is predominantly single exponential, and polydispersity in chromatin fragment length may be neglected in the present analysis. The angular and concentration dependence of $C(\tau)$ is represented in a plot of q^2D vs. q^2 in Figure 1B. This plot is linear with a slope D and extrapolates through the origin. This indicates that the relaxation of $C(\tau)$ contains negligible contributions from processes other than translational diffusion, such as rotational diffusion, chemical reaction, or internal modes. If polydispersity were significant for macromolecular complexes of the dimensions reported herein, then this curve should appear concave upward with an apparent negative intercept. Finally, the translational diffusion coefficients were independent of chromatin concentration over the range studied.

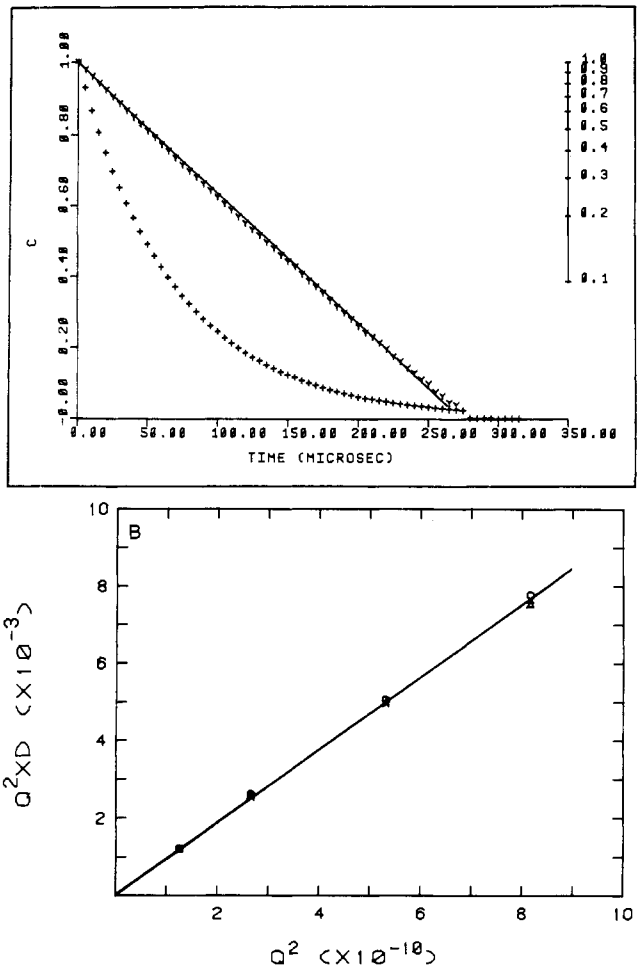

FIGURE 1: Quasi-elastic light scattering measurements of S chromatin in 0.15 M NaCl, 10 mM Tris-HCl, and 1 mM EDTA, pH 7.8, at 20 °C. (A) Homodyne autocorrelation function, obtained with a channel time of 5 μs at a scattering angle of 120° and chromatin concentration of 0.271 mg/mL: (Y) logarithmic ordinate on right; (+) linear ordinate on left. (B) Angular and concentration dependence: (○) 0.060, (×) 0.151, and (△) 0.271 mg/mL.

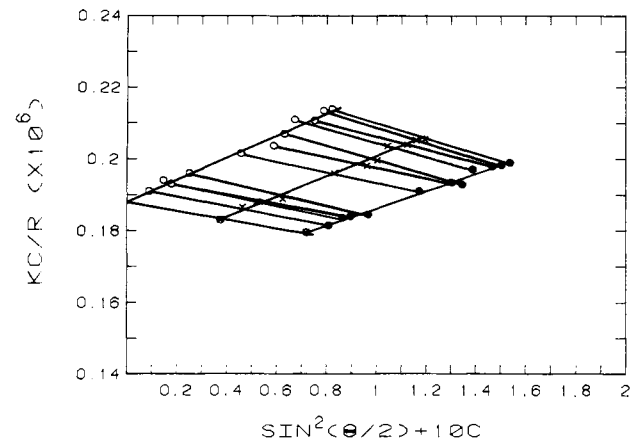

FIGURE 2: Zimm plot of static light scattering measurement of I chromatin in 0.08 M NaCl, 10 mM Tris-HCl, and 1 mM EDTA, pH 7–8, at 20 °C: (●) 0.073 mg/mL; (×) 0.037 mg/mL.

A typical Zimm plot is shown for I chromatin in Figure 2. The data were extrapolated separately to $c = 0$ and to $\theta = 0$. These two extrapolated curves intersect at a common intercept on the Kc/R axis which represents the reciprocal weight-average molecular weight. The slope of the curve extrapolated to $c = 0$ yields R_G [see Tanford (1961)].

A check on systematic errors in the acquisition and analysis of total intensity light scattering data was made by comparisons of M values determined from Zimm plot analysis and M values determined by the Svedberg equation. Translational diffusion coefficients of various S chromatin fractions in 0.15 M NaCl, 10 mM Tris-HCl, and 1 mM EDTA, pH 7.5, were determined by QLS. Sedimentation coefficients were determined on the same samples in the analytical ultracentrifuge. A single sedimenting boundary was observed for all samples. These data are presented in Table I. Molecular weights obtained by these two different methods agreed to within a few percent.

Table I: Comparison of S Chromatin Molecular Weights Determined by Light Scattering and Sedimentation/Diffusion

sample	$D_{20,w}$	$s_{20,w}$ (s)	M^a (Svedberg)	M^b (Zimm)
1	1.02×10^{-7}	61.1	4.05×10^6	3.92×10^6
2	0.96×10^{-7}	74.0	5.19×10^6	5.33×10^6
3	0.90×10^{-7}	84.9	6.30×10^6	5.93×10^6
4	0.60×10^{-7}	114.2	12.35×10^6	12.00×10^6

[a] Molecular weight determined by the Svedberg equation: $M = (s_{20,w}/D_{20,w})[RT/(1 - \bar{v}_p)]$ where $\bar{v}_p$ was taken to be 0.64 (McGhee et al., 1980a). [b] Molecular weight determined by total intensity light scattering as described in the text and Figure 2.

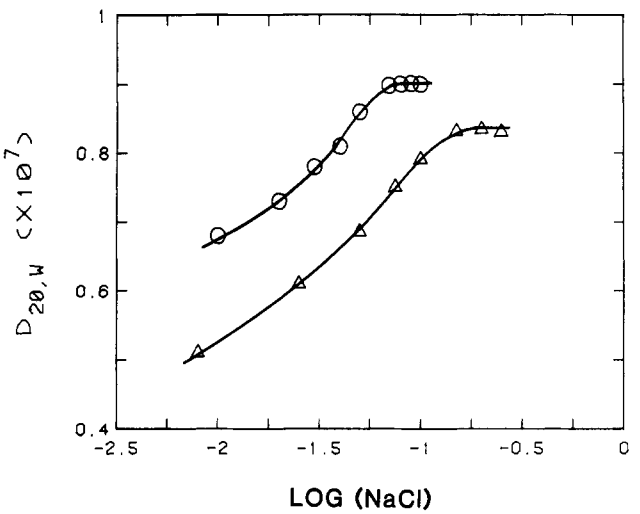

FIGURE 3: Translational diffusion coefficient, $D_{20,w}$, of S chromatin ($\bar{N} = 26$) and I chromatin ($\bar{N} = 19.0$) as a function of NaCl concentration. All samples contained 10 mM Tris-HCl and 1 mM EDTA, pH 7.5.

The effect of ionic strength on the translational diffusion of S and I chromatins is presented in Figure 3. Fractionated S and I chromatin samples were dialyzed against 10 mM Tris-HCl and 1 mM EDTA, pH 7.5, containing various concentrations of NaCl. D of both S and I chromatins increased with increasing salt concentration until plateaus were reached at higher ionic strengths. Excess light scattering intensities indicated no ionic strength dependent changes in M (data not shown). Therefore, changes in D probably reflect changes in the degree of compaction. I chromatin was maximally compact between 0.07 and 0.10 M NaCl, just before precipitation, while S chromatin reached its limit between 0.15 and 0.25 M NaCl. Some of the change in D may also be due to electrolyte friction (Schurr, 1980). This possibility is examined under Discussion.

Preparations of S and I chromatins were fractionated according to size by gel filtration chromatography under conditions where these structures were maximally condensed: 0.08 M NaCl for I chromatin and 0.15 M NaCl for S chromatin [see Fulmer & Bloomfield (1981) for elution profiles and experimental details]. Radii of gyration and hydrodynamic

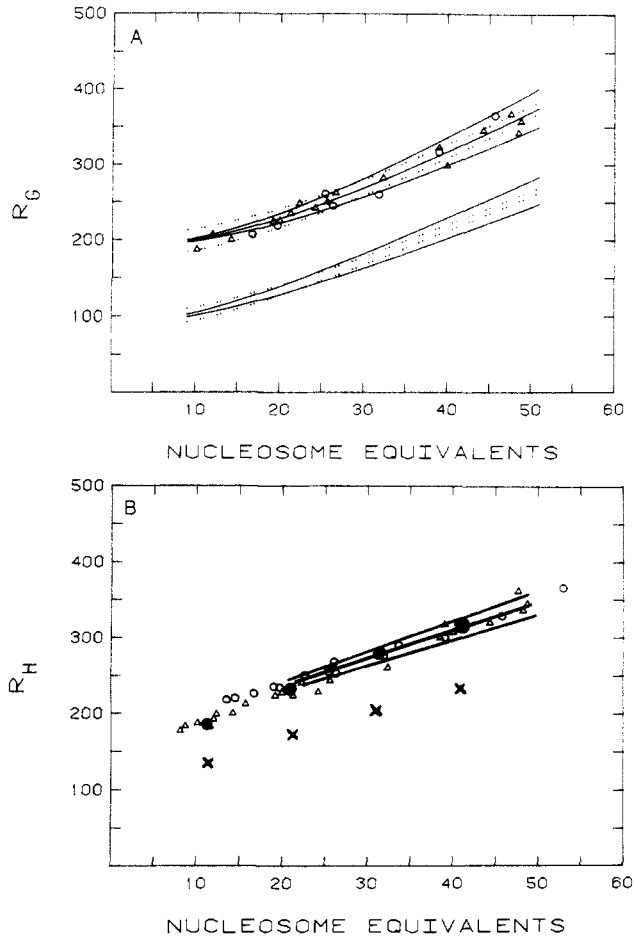

FIGURE 4: (A) Radius of gyration R_G of S chromatin in 0.15 M NaCl (Δ) and I chromatin in 0.08 M NaCl as a function of fragment length. Upper, middle, and lower solid lines (upper set) correspond to theoretical expectations for helical structures with a 24-, 22-, and 20-Å rise/residue, respectively, and r = 190 Å. Upper and lower dotted lines (upper set) represent r = 200 and 180 Å, respectively, with a 22-Å rise/residue. The lower set of solid and dotted lines was generated for close-packed solenoidal structures (see Discussion). (B) Hydrodynamic radius R_H of S chromatin in 0.15 M NaCl (Δ) and I chromatin in 0.08 M NaCl (O) as a function of fragment length. Solid circles (●) are results of hydrodynamic calculations (Garcia de la Torre & Bloomfield, 1977) based on the above helical parameters. Solid lines correspond to calculations for prolate ellipsoids. Large (×) symbols are results of hydrodynamic calculations based on compact solenoidal parameters (see Discussion).

radii were determined over a range of molecular weights which correspond to 8 through 53 nucleosome equivalents ($\bar{N}$). These data are presented in Figure 4A,B and represent samples from three independent preparations. Chromatin samples remained stable for 10–14 days after the preparation of nuclei when stored under these conditions at 4 °C. Thus, these data are considered to represent a stable structural configuration of chromatin oligomers in aqueous solution devoid of divalent cations.

The following strategy was employed to analyze the data in Figure 4A,B. Data in Figure 4A [$R_G(\bar{N})$] were fit to a continuous helix by varying d, the rise per nucleosomal residue along the helix (z) axis, and r, the radius of the helix measured from the helix axis to the center of a nucleosomal residue lying in the x–y plane. The number of nucleosomes per helical turn, n, was calculated for a helix of contiguous spherical nucleosomes of identical diameters. These parameters were then used to generate helical assemblies of subunits which were analyzed by the method of Garcia de la Torre & Bloomfield (1977) to yield $R_H(\bar{N})$. Consideration of close-packed "superbead-like" structures will be deferred until the Discussion.

The average radius of gyration for an assembly of identical mass elements of mass m_i, each located a distance r_i from the center of mass, is defined as

$$R_G^2 = \sum_{i=1}^{\bar{N}} m_i r_i^2 / \sum_{i=1}^{\bar{N}} m_i = \frac{1}{\bar{N}} \sum_{i=1}^{\bar{N}} r^2$$

The center of mass was taken as the position on the z axis which corresponded to the middle, $(\bar{N}+1)/2$, residue for odd $\bar{N}$. If the centers of identical nucleosomes of mass m_i are placed r_i from the center of mass, then

$$R_G = [r^2(1 + 2m) + (d^2/3)m(m + 1)(2m + 1)]^{1/2}$$

where $m = (\bar{N}-1)/2$. The derivation of this equation neglects the finite size of nucleosomal subunits. However, for $\bar{N}$ = 11, the above equation underestimates the exact solution by only 2%, and the discrepancy diminishes as $\bar{N}$ increases.

Values of R_G were calculated for odd $\bar{N}$ ($9 < \bar{N} < 51$) as a function of r and d by a Tektronix 4052 graphics system. The best agreement between the experimental data and theoretical curves was determined by visual inspection and resulted (upper set of solid and dotted curves) in r = 190 Å and d = 22 Å (middle solid curve in Figure 4A). The upper and lower solid curves represent d = 24 Å and d = 20 Å, respectively, with r held constant at 190 Å. The upper and lower dotted curves represent r = 200 Å and r = 180 Å, respectively, with d held constant at 22 Å. The lower set of solid and dotted curves corresponds to close-packed solenoidal structures (see Discussion). Thus, experimental $R_G(\bar{N})$ data are consistent with a helical model for oligonucleosomes where r = 190 ± 10 Å and d = 22 ± 2 Å over a range of N from 10 to 50 nucleosomes.

Oligonucleosome helixes are assumed to consist of contiguous (Sperling & Tardieu, 1976) spherical nucleosomal subunits of diameter l. A subunit diameter of l = 106 Å was calculated from known dimensions of the nucleosome core particle with additional allowances made for the presence of linker DNA and lysine-rich histones. The contour length of one turn of a continuous helix, is L_c,

$$L_c = (P^2 + 4\pi^2 r^2)^{1/2}$$

where P is the helix pitch and $P = nd$. Since $L_c = nl$, then

$$n = 2\pi r/(l^2 - d^2)^{1/2}$$

Thus, for r = 190 Å, d = 22 Å, and l = 106 Å, our model for the nucleosomal helix contains n = 11.5 nucleosomes/turn with a pitch of 253 Å.

These parameters were used to generate helixes of $\bar{N}$ = 11, 21, 31, and 41 subunits which were analyzed by the method of Garcia de la Torre & Bloomfield (1977). The results of these analyses are plotted as large solid circles in Figure 4B. Agreement with the experimental data is excellent. The lines running through the data for $20 < \bar{N} < 50$ were generated for solid cylinders which were modeled as polate ellipsoids of equivalent length and volume. Cylinder lengths, L, were taken as $L = \bar{N}d$. Upper, middle, and lower solid curves represent d = 24, 22, and 20 Å, respectively, with r = 200 Å. The upper and lower dotted curves represent r = 210 and 190 Å, respectively, with d = 22 Å. The significance of these calculations will be discussed below.

S and I chromatin fragments were fixed with glutaraldehyde, negatively stained with uranyl acetate, and examined by electron microscopy. Images of I chromatin fragments ($37 < \bar{N} < 53$) which were fixed in 0.08 M NaCl buffer are shown in Figure 5. These fragments displayed a rodlike appearance with diameters around 330 Å. Although internal morphologies

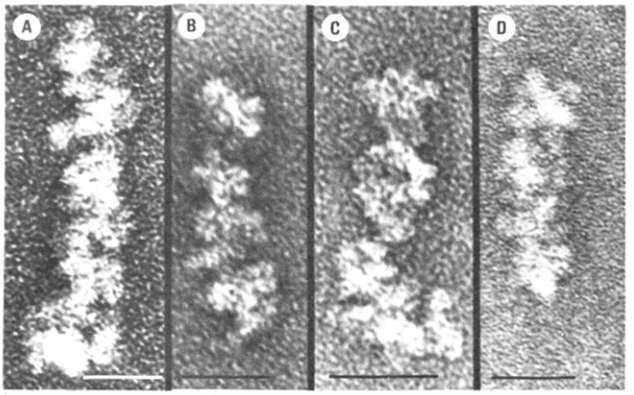

FIGURE 5: Negatively stained (uranyl acetate) images of I chromatin fragments ($37 \leq \bar{N} \leq 53$) fixed in 0.08 M NaCl buffer with glutaraldehyde. The solid bars at the bottom of each frame represent 500 Å.

are often suggestive of a helical structure, the present resolution precludes definitive interpretation. S chromatin fragments ($40 \leq \bar{N} \leq 50$) which were fixed in 0.15 M NaCl buffer displayed similar morphological characteristics.

Discussion

We have previously shown (Fulmer & Bloomfield, 1981) that bulk chromatin isolated from chicken erythrocyte nuclei can be fractionated into two major classes on the basis of solubility at physiological ionic strengths. Approximately 40% of the total genome, S chromatin, was shown to be completely soluble between 0.01 and 0.25 M NaCl while the remaining 60% of the total genome, I chromatin, precipitated from solutions containing NaCl concentrations above 0.1 M. The structural basis for these different solubilities is unknown at present. A referee has suggested that H1 mobility may be involved (Lasters et al., 1981). However, we have no evidence for this idea at present. The present study reports the effects of NaCl on higher order folding of these two different classes of chromatin chains.

Translational diffusion coefficients of both S and I chromatins increased with increasing NaCl concentration until plateaus were reached at the higher ionic strengths (Figure 3). The plateau in D for I chromatin was located between 0.07 and 0.10 M while S chromatin displayed similar characteristics between 0.15 and 0.25 M. Excess light scattering intensities indicated no change in molecular weight. Thus, these data represent changes in the intramolecular folding of S and I chromatin fragments. These results may be compared to those of a recent sedimentation study on bulk chromatin isolated from rat liver nuclei by Butler & Thomas (1980). They observed a simple power law dependence of the sedimentation coefficients (s) upon ionic strength I, $s = \alpha I^n$, where $n = 0.16 \pm 0.01$ for $13 < \bar{N} < 30$ and $5 <$ [NaCl] < 85 mM. Our preplateau data show a power law dependence with $n = 0.17$. Thus, the bulk preparations of the Cambridge workers displayed NaCl concentration effects similar to those of our own preparations of fractionated chromatin. One notable difference between the data reported by Butler & Thomas (1980) and our own is the absence of a plateau in S vs. I in the former. This behavior is expected, however, since their bulk chromatin preparations probably contained a mixture of S and I chromatins which might obscure the plateau observed for I chromatin between 0.07 and 0.10 M NaCl.

Electrolyte friction (Schurr, 1980) may be responsible for some of the decrease in D seen at low salt concentration. Application of the theory quantitatively requires knowing the net charge on the nucleosome. In the absence of a firm value, we have assumed 10–20 charges, corresponding to 90–95% neutralization of the DNA phosphate charge by bound histones and other counterions. The calculations (not shown) clearly indicate that electrolyte friction can account for a substantial fraction of the observed effect. However, if the charge/nucleosome is large enough (between 10 and 20 charges) to bring D down to its observed low-salt value, then the high-salt D is significantly less than observed, and the plateau is not predicted to appear until I is significantly greater than 0.1 M. Furthermore, chromatin fibers cross-linked in a stabilized compact configuration (Lee et al., 1981) do not show a significant dependence of D on I between 10 and 0.5 mM. Since this preparation should differ from ours in flexibility but not in charge, this argues that chain expansion is the major factor in the decrease of D.

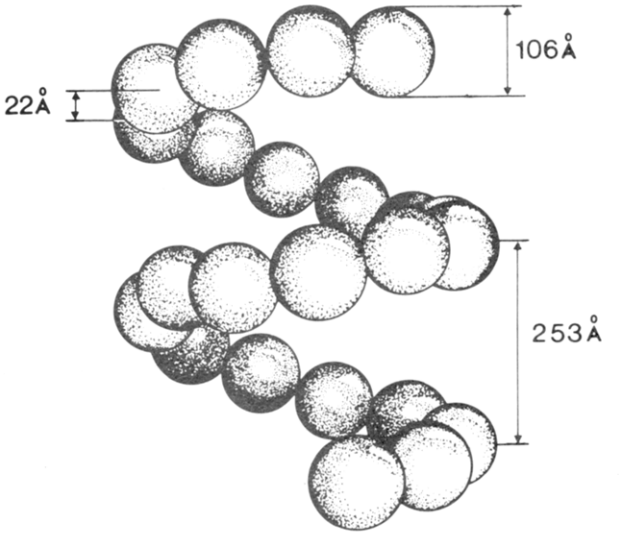

FIGURE 6: Schematic of extended helix model derived from data in the present study. Helical parameters are as follows: 22-Å rise/residue, 190-Å radius from helix axis to center of nucleosomal residue, 240-Å outer radius, 253-Å pitch, 11.5 nucleosomal residues/turn.

It is worthwhile considering whether D of the completely stretched out oligonucleosome is low enough to account for the low-salt observations. We have made such calculations by using a cylindrical model of length $\bar{N}l$ and diameter l. Using the modified Broersma (1981) equations, with l = 106 Å, we find for $\bar{N} = 26$ $D_{calcd} = 5.11 \times 10^{-8}$ cm^2/s and $D_{obsd} = 5.1 \times 10^{-8}$ cm^2/s; for $\bar{N} = 19$, $D_{calcd} = 6.28 \times 10^{-8}$ cm^2/s and $D_{obsd} = 6.8 \times 10^{-8}$ cm^2/s. Thus, it appears that oligomers can stretch sufficiently to account for the low-salt data.

Our efforts have been focused on characterizing higher order folding of both S and I chromatin fragments under ionic conditions which allow maximal compaction. Thus, we have studied I chromatin at an ionic strength of 80 mM and S chromatin at 0.15 M NaCl. Series of fragment lengths were obtained by gel filtration of S and I chromatins under these ionic conditions. These methods have allowed us to isolate a wide range of average fragment lengths ($8 \leq \bar{N} \leq 50$) which displayed minimal polydispersity as judged by QLS (Figure 1). Determination of hydrodynamic radii by QLS and both radii of gyration and molecular weight by static light scattering (Figure 4) has allowed us to construct a single model for the folding of chromatin fragments which is in excellent agreement with this rather wide range of fragment lengths for both S and I chromatins. This model is shown schematically in Figure 6. It is an extended helix of contiguous spherical nucleosomes with a rise per nucleosomal residue of 22 Å, helix pitch of 253 Å, outer diameter of 480 Å, and 11.5 nucleosomal residues/helical turn.

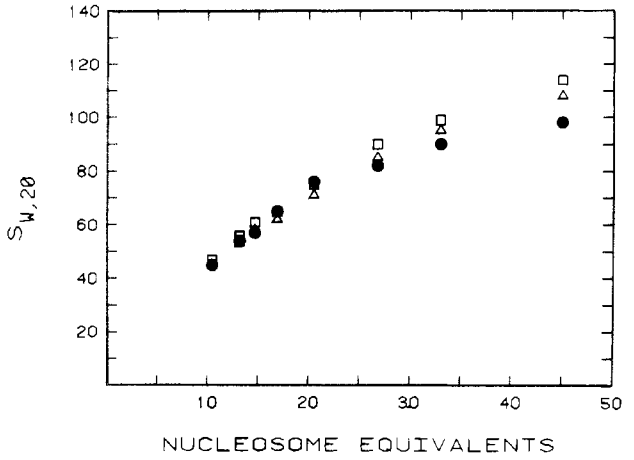

FIGURE 7: Comparison of sedimentation coefficients reported by Butler & Thomas (1980) (●) for chromatin fragments in 0.085 M NaCl to those derived from the hydrodynamic data in Figures 3 and 4B for I chromatin (□) and S chromatin (△) as a function of fragment length.

Chromatin fibers of metaphase chromosomes and interphase nuclei display predominantly ~250–300-Å diameters in negatively stained electron micrographs (Brasch, 1976; Ris & Kubai, 1970). Finch & Klug (1976) have proposed that this level of chromatin organization is achieved by helical coiling of a chain of contiguous nucleosomes (100-Å fiber) into a close-packed helix or solenoid of diameter ~300 Å, with 6–7 nucleosomes/turn and a central hole of ~100-Å diameter. Results of various solution studies have supported such a model (Suau et al., 1979; Campbell et al., 1978), as do electrooptical studies of chromatin fragments in solutions containing low concentrations of Mg^{2+} (McGhee et al., 1980a,b; Lee et al., 1981). X-ray studies of chromatin fibers were also consistent with this type of model (Sperling & Klug, 1977). However, Carlson & Olins (1976) have calculated that a large variety of spherical nucleosomal arrays can display the characteristic X-ray maxima of chromatin preparations. Our data for chromatin fibers in aqueous solutions of NaCl which are devoid of divalent cations are *not* compatible with such close-packed solenoidal structures. The lower set of solid and dotted curves in Figure 4A were generated for solenoidal structures containing 6 and 7 nucleosomes/turn, 110-Å pitch with 90-Å helix axis to bead center radius (upper and lower solid lines). Upper and lower dotted lines correspond to 6.5 nucleosomes/turn, 110-Å pitch with 100- and 80-Å radii. Hydrodynamic radii were generated for $\bar{N}$ = 11, 21, 31, and 41 for a solenoidal helix containing 6 nucleosomes/turn, 106-Å nucleosome diameter, and 110-Å pitch with 100-Å helix axis to bead center radius by the method of Garcia de la Torre & Bloomfield (1977) and are represented as large X's in Figure 4B. These parameters lead to an outer diameter of 306 Å.

Butler & Thomas (1980) have recently reported sedimentation studies of bulk chromatin preparations as a function of ionic strength and chromatin fragment length. Their results were interpreted as a continuous pattern of helical condensations resulting in close-packed solenoidal structures at the higher ionic strengths studied (0.080–0.125 M NaCl). However, quantitative analyses of their frictional data were not presented. These data are compared to our own in Figure 7. Sedimentation coefficients for bulk chromatin oligomers with $10.5 \leq \bar{N} \leq 26.8$ at the highest ionic strength reported (85 mM NaCl) were taken from Figure 4a of Butler & Thomas (1980). Data for $\bar{N}$ = 33 and 45 were taken from Figure 1 of Thomas & Butler (1980) for 85 mM NaCl. Sedimentation coefficients of S and I chromatin fragments were calculated from measured translational diffusion coefficients and molecular weights by the Svedberg equation. Since I chromatin displayed maximal compaction at 85 mM NaCl, D was derived from direct interpolation of the data in Figure 4B. Translational diffusion coefficients of S chromatin fragments were estimated by scaling those interpolated for I chromatin according to the ratio in frictional behavior of S chromatin at 0.085 and 0.15 M NaCl (Figure 3). Since Thomas and Butler were most likely studying a mixture of S and I chromatins, we would expect to find their data falling between the limits of our own S and I chromatin data. Indeed, such behavior is observed for $\bar{N} \leq 20$. However, the larger chromatin fragments of Butler & Thomas (1980) displayed more frictional resistance than those reported in the present study. The reason for this is not understood. The major point here is that our own chromatin preparations appear to be hydrodynamically identical with those of the Cambridge group. However, quantitative analysis of both hydrodynamic radii and radii of gyration indicates that close-packed solenoidal structures are not present in dilute aqueous solutions of NaCl which are devoid of divalent cations.

Table II: Comparison of Fractionated Chromatin Dimensions Obtained by Electron Microscopy and Light Scattering

$\bar{N}^a$	diameter[b] (EM) (Å)	length[b] (EM) (Å)	diameter[c] (LS) (Å)	length[c] (LS) (Å)
37–53	333 ± 21		480 ± 20	
25	331 ± 23	390 ± 29	480 ± 20	550 ± 50

[a] Weight-average number of nucleosomes per chromatin chain as determined by light scattering. [b] Mean dimensions (± standard deviation) of chromatin chains determined from electron micrographs. [c] Mean dimensions (± standard deviation) of chromatin chains determined by light scattering.

Since the bulk of experimental support for the 300-Å solenoidal model has been derived from electron micrographs of negatively stained or shadowed chromatin fragments (Finch & Klug, 1976; Thoma et al., 1979), we have performed parallel light scattering and electron microscopic studies on several S and I chromatin fractions under ionic conditions leading to maximal compaction. Comparisons of fractionated chromatin dimensions obtained by electron microscopy and light scattering are summarized in Table II. Long chromatin fragments ($37 < \bar{N} < 53$), such as those shown in Figure 5, displayed approximately 330-Å diameters in negatively stained images. However, light scattering studies on these fragments in solution indicated that the outer diameters should lie around 480 Å. Smaller chromatin fragments, e.g., $\bar{N}$ = 25, displayed similar discrepancies in molecular dimensions and appeared somewhat globular. Both lengths and diameters of these chromatin fragments were reduced by approximately 30% when electron micrographs are compared to solution dimensions. The degree of shrinkage is most likely sensitive to the details of sample preparation.

A recent electron microscopic study (Lepault et al., 1980) on nuclei and chromatin has employed freeze–fracture techniques which are believed to eliminate potential artifacts due to fixation, dehydration, and staining which plague the more classical electron microscopic studies such as those cited and reported above. These investigators found that the majority of chromatin was organized into 400–500-Å diameter fibers. Fiber diameters around 250–300 Å were rarely observed. Their observations were also incompatible with a repeating superbead structure of chromatin.

Other authors have previously proposed extended helical structures for chromatin fragments in aqueous solution. Although these models are not in quantitative agreement with

Table III: Comparison of Observed and Calculated Sedimentation Coefficients of Putative Globular Nucleosomal Particles

sample	s_{calcd}[a] (s)	s_{obsd}[b] (s)	s_{est}[c] (s)
1	144	97	95
2	153	112	102

[a] Sedimentation coefficients calculated for spheres of the masses and diameters reported in Table II of Jorcano et al. (1980). A value of 0.64 was used for $\bar{\nu}_p$ (McGhee et al., 1980a). [b] Experimental sedimentation coefficients reported in Table II of Jorcano et al. (1980). [c] Sedimentation coefficients interpolated from the data in Figure 4B and the molecular weights reported in Table II of Jorcano et al. (1980).

our own, these differences may lie in the different ionic strengths employed. Bram and co-workers (Bram et al., 1975, 1977) have studied chromatin fragments around 10^{-2} M ionic strength by small-angle neutron scattering. Their data were modeled as extended helixes of 250–300-Å diameter with 400–500-Å pitch. Elaborate hydrodynamic modeling of translational frictional data on chromatin fragments around 10^{-2} M ionic strength by Schmitz and co-workers [Shaw & Schmitz (1979) and references cited therein] has also produced extended helixes of 370-Å diameter with 250-Å pitch. Light scattering and neutron diffraction data of Campbell et al. (1978) and Suau et al. (1979), respectively, also supported the existence of extended helixes under these rather low ionic strength conditions. However, these latter authors, as well as Butler & Thomas (1980), have suggested that higher ionic strengths (~0.1 M) induce the contraction of extended helixes to close-packed solenoidal structures. Our results are not in agreement with this interpretation.

A second class of models, which has received considerable recent attention, represents the chromatin fiber as a discontinuous assembly of compact globular clusters of 6–14 nucleosomes called superbeads (Hozier et al., 1977; Renz, 1979; Renz et al., 1977; Stratling et al., 1978; Itkes et al., 1980; Jorcano et al., 1980; Stratling & Klingholz, 1981). Our data on S and I chromatin for $8 < \bar{N} < 50$ did not reveal any periodic discontinuities which might arise from multiples of discrete globular clusters of nucleosomes. Similar conclusions were reached by Butler & Thomas (1980). Moreover, this range of fragment lengths could be modeled as prolate ellipsoids (Figure 4B) which displayed a constant hydrodynamic volume per nucleosomal subunit of 27.6×10^5 Å^3. The hydrodynamic volume of an individual nucleosome is estimated to be around 6.3×10^5 Å^3. This factor of 4–5 in hydrodynamic volumes casts serious doubt on the existence of close-packed structures.

A recent study by Jorcano et al. (1980) has reported globular particles of oligonucleosomes containing a total of approximately 25 nucleosomes. These particles appeared spherical with ~300-Å diameters when negatively stained for electron microscopy. Table II of Jorcano et al. (1980) reported sedimentation coefficients, molecular weights, and particle diameters (via electron microscopy). We have reanalyzed these data in Table III. Particle diameters and molecular weights were used to calculate expected sedimentation coefficients for these authors' chromatin samples (column 2 of Table III). One can see that they are significantly higher than the measured sedimentation coefficients reported in Table II of Jorcano et al. (1980) (column 3 of Table III). Molecular weights reported in Table II of Jorcano et al. (1980) were used to estimate translational frictional coefficients by interpolation of our data in Figure 4B. Sedimentation coefficients were then calculated by the Svedberg equation and are presented in column 4 of Table III. The agreement between these calculated sedimentation coefficients and those measured by Jorcano et al. (1980) is very good. Thus, the higher order folding of chromatin preparations in aqueous solution studied by Jorcano et al. (1980) appears to be very similar to that observed in the present study. The compact globularity was probably a result of specimen preparation for electron microscopy. Such shrinkage was observed in our own preparations (Table II).

Our model must also be compared to a third possible molecular configuration, the random coil. Filson & Bloomfield (1968) analyzed experimental sedimentation data on polysomes in aqueous solution which had been obtained by other workers. Although the available data only extended to 12 ribosomes/polysome, they were able to narrow the number of possible models to two general classes, either extended helixes or random coils with excluded volume effects and small attractive interribosomal interactions. Translational friction data alone were not able to distinguish between these models. It is unclear if the additional constraints imposed by radius of gyration data can distinguish between these two models, since Monte Carlo modeling to calculate R_G would be required. However, we feel that the rodlike shapes of large chromatin fragments observed in the electron microscope (Figure 5) support a helical model over any random configuration.

Extended helical structures might not be regarded as particularly stable structures in aqueous solution. Their possible existence implies rather strong and directional interactions between neighboring nucleosomal subunits which could arise from a somewhat tilted or wedge-shaped nucleosome structure. Wedge-shape core particles have been discussed by Finch et al. (1977) and Dubochet & Noll (1978). One would expect extended helical structures to be very sensitive to the microenvironment. Thus, it is not surprising to find discrepancies in dimensions derived from solution studies and electron microscopy. More compact structures may exist in solutions containing divalent cations, or in the very crowded, concentrated solutions required for neutron scattering. Extended helical structures are inherently soft and may be readily switched to other modes of packing by subtle manipulation of the microenvironment. It is also interesting to note that gyres of two or more extended helixes may be intercalated to share a common helix axis. This possibility is particularly intriguing when one considers various higher order multifiber modes of packing a single fiber by folding it back and forth upon itself, such as the models discussed by Mullinger & Johnson (1980). In summary, the four main points of this paper are the following.

(1) The two different classes of bulk chromatin (Fulmer & Bloomfield, 1981) display ionic strength dependent contractions to folded structures which are hydrodynamically indistinguishable (Figure 4). These conformational transitions occur over different ranges of ionic strength for S and I chromatins (Figure 3). Molecular dimensions become maximally compact and insensitive to ionic strength between 0.07 and 0.10 M for I chromatin and 0.15 and 0.25 M for S chromatin. I chromatin is extensively aggregated over this latter range of ionic strengths (Fulmer & Bloomfield, 1981).

(2) Our chromatin preparations are hydrodynamically indistinguishable from preparations described by Butler & Thomas (1980) (Figure 7). These authors support close-packed solenoidal structures for chromatin around 0.1 M ionic strength. However, both hydrodynamic radii and radii of gyration collected for $8 \leq \bar{N} \leq 50$ indicate that such structures are most likely absent from aqueous solutions containing NaCl but devoid of divalent cations.

(3) Our chromatin preparations are also very similar hydrodynamically to preparations described by Jorcano et al. (1980) (Table III). These authors supported discrete globular clusters of close-packed nucleosomes. Again, both hydrodynamic radii and radii of gyration collected for $8 \leq \bar{N} \leq 50$ indicate that close-packed clusters of nucleosomes are most likely absent from aqueous solutions containing NaCl but devoid of divalent metal ions.

(4) Analysis of our own results (Table II), as well as results presented by Jorcano et al. (1980) (Table III), indicates that chromatin fragments can undergo considerable shrinkage when prepared for classical electron microscopic studies. Chromatin studies employing freeze–fracture techniques are in better agreement with our solution data (Lepault et al., 1980).

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