Quasielastic Light Scattering by Biopolymers. Conformation of Chromatin Multimers*

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Abstract

Chicken erythrocyte chromatin was partially digested with micrococcal nuclease and separated into multimeric subunit fractions by gel permeation chromatography. The fractions were characterized by their Svedberg constant, diffusion coefficient, circular dichroism, and electrophoresis pattern of the extracted DNA. The molecular weight dependence of the sedimentation coefficient was found to be $S_{20,w} = .011 \times M^{.554}$. The molecular weight dependence of f/f_o is best represented in the Kirkwood theory by either a helical superstructure or a flexible coil with attractive interactions between nucleosome units. The dimer calculations of f/f_o suggest that the core particles are separated by spacer regions which contribute up to ~20% of the frictional properties of the molecule.

Introduction

Partial nuclease digestion of chromatin from mammals (1-3), yeast (4), plants (5) and other eukaryotes (6, 7) suggests that nuclease resistant regions are periodically organized along the chromatin strand. Kornberg (8) and Van Holde et al. (9) independently proposed models in which a short DNA strand and two each of the histones H2A, H2B, H3 and H4 comprise the repeat unit. Chemical evidence (10-14) suggests that 40-60 base pairs of nuclease accessible DNA serve to link core particles, i.e., a DNA-histone complex in which ~140 base pairs of DNA is wrapped around the eight-histone complex (9, 15, 16), thereby giving rise to a "beads-on-a-string" structure as observed by electron microscopy (17-20).

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We report herein the dependence of hydrodynamic properties of chromatin multimers on molecular weight. Quasielastic light scattering and sedimentation methods were used to obtain, respectively, the diffusion (D) and Svedberg (S) coefficients employed in the functional analysis. The Kirkwood theory (27) for hydrodynamic interaction between friction beads on a string was used to compute f/f_o values for rigid rod, regular planar polygon, helix, and, using the results of Filson and Bloomfield (21), the random flight polymer in a free space, tetrahedral and hexagonal lattices. The results of our investigations suggest that the supramolecular structure of nucleosomes in solution is compact, obtaining conformations of either a helix or flexible coil with attractive interactions between core particles.

Experimental

The preparation and isolation of chicken erythrocytes were described previously (11, 12). Digestion of the nuclei by micrococcal nuclease (Worthington) was carried out at 37°C and terminated by making the solution 10mM in EDTA and cooling on ice. The nuclei were centrifuged at 12000xg for 15 minutes, resuspended in 10 ml of 10mM Tris·HCl, pH 7.5, 0.7mM EDTA and disrupted 30 seconds at medium setting on a Virtis homogenizer. Nuclear debris was pelleted at 10,000xg for 15 minutes and the supernatant was made 7% in sucrose and applied to a Bio-Rad A-5m column, 90 x 2.5 cm, equilibrated with 10mM Tris·HCl, 0.7mM EDTA, pH 7.5 at 5°C. The dimer fraction was further purified by isopycnic sucrose gradient centrigugation

Sedimentation coefficients were obtained on a Beckman Model E ultracentrifuge at 30,000 rpm. Diffusion coefficients were determined by quasielastic light scattering methods on the facility previously described (22-24). Samples were gravity-flow filtered into the scattering cells at 5°C and temperature control throughout the data collection interval was effected by circulating ice water in a temperature jacket similar to that previously described (23). The single exponential decay autocorrelation function, computed with a Saicor 43A autocorrelator operating in the clip mode, has a homodyne decay constant $1/\tau$ given by (25)

$$1/\tau = 2D(4\pi n/\lambda_o)^2 \sin^2(\theta/2) \qquad (1)$$

where D is the diffusion coefficient, n is the index of refraction, λ_o = 488 nm,

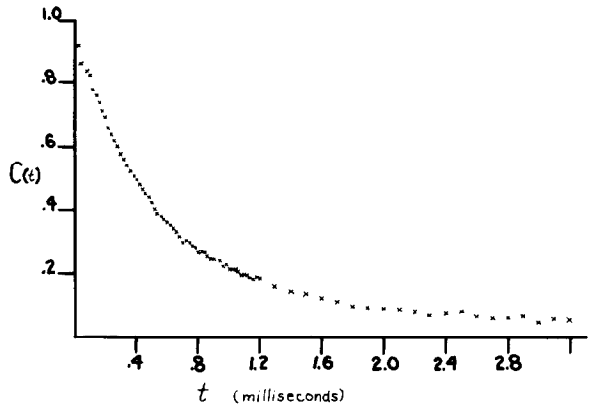

Figure 1

Representative Autocorrelation Function of Chromatin Multimers.

The autocorrelation functions C(t) in the present study were analyzed by least-squares analysis of a single exponential decay with baseline,

$$C(t) = A \exp(-2DK^2 t) + B$$

where $K = (4\pi n/\lambda_0)\sin(\theta/2)$ (cf. text for definition).

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and θ is the scattering angle. ($40 \leq \theta \leq 70$). A representative curve is given in Figure 1.

Data Representation

The molecular weight was computed from the Svedberg equation,

$$M = (S/D)RT \times 10^{-13}/(1-\bar{v}\rho) \quad (2)$$

where S is in Svedbergs and $\bar{v}\rho = .68$. A plot of ln S vs ln M is given in Figure 2 for the chromatin multimers and native DNA (26). The empirical relationships for the two sets of data are

$$S_{20} = 0.011\ M^{.554} \qquad \text{(multimers)} \quad (3)$$

and

$$S_{20} = 0.163\ M^{.280} \qquad \text{(native DNA)} \quad (4)$$

The molecular weight dependence of the friction factor ratio f/f_o, using the Einstein relationship

$$f = kT/D \quad (5)$$

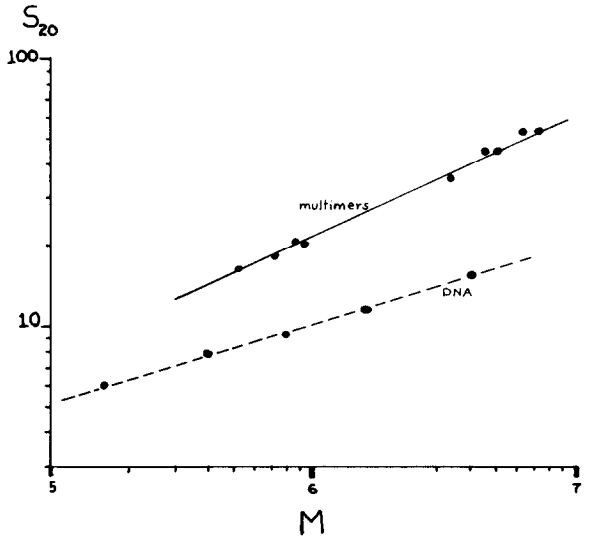

Figure 2

Molecular Weight Dependence of S_{20} for Chromatin Multimers and Native DNA

The molecular weight of the multimers were computed from the diffusion and sedimentation coefficients for column fractions (in 10mM Tris· 0.7mM EDTA, pH 7.5) of partial nuclease digested chicken erythrocyte chromatin (containing lysine rich histones H1 and H5). The native DNA values were reported by Record, Woodbury and Inman (26).

(——) $S_{20} = .011\ M^{.554}$

(----) $S_{20} = .163\ M^{.280}$

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and Stoke's equation

$$f_o = 6\pi\eta R_s = 6\pi\eta\left[\frac{3}{4\pi}\frac{\bar{v}M}{N}\right]^{1/3} \qquad (6)$$

where η is the solvent viscosity and N is Avogadro's number, was compared with the theoretical expression of the Kirkwood theory (27)

$$\frac{f}{f_o} = \frac{nR}{R_s\left(1 + \frac{R}{n}\sum_{i\neq j} \langle 1/R_{ij}\rangle\right)}$$

where R is a friction bead radius, n is the number of beads per chain, $\langle 1/R_{ij}\rangle$ is the average distance between centers of beads i and j, and R_s is defined in Eq. (6)

Results and Discussion

The power dependence of S_{20} on molecular weight suggests that the chromatin multimers (a = .554) exhibit a more compact conformation than that of native DNA (a = .280), which is closely represented as a rigid rod at these molecular weights (28). The three-dimensional conformation of the polynucleosomes is inferred from f/f_o when compared with model structures employing Eq. (7). Since several unknown parameters are involved in describing the macrostructure (i.e., center-to-center distance B of adjacent beads, R, R_s, pitch, and fiber diameter for a helical conformation), the parameters R and B are first estimated from the dimer fraction, where R_s = 52Å (cf. Eq. 6) for the molecular weight of 5.3×10^5 (S_{20} = 16.2). The ratio f/f_o = 1.85 for the dimer yields a value of R = 73Å in the contiguous bead model (B = 2R). This value is much larger than the reported estimates of the core particle radius, ranging from 50-55Å (9, 11, 15, 17, 18, 29-31). We interprete this apparent inconsistency as physical evidence in the support of spacer regions between core particles in solution. If we fix the value of B at 236Å, estimated from a core particle radius of 50Å and a spacer region of 40 x 3.4Å = 136Å, we calculate an "effective" friction bead radius R' of R' = 62Å. The hydrodynamically equivalent dimer, therefore, corresponds to "effective" friction beads of radius R' = 62Å connected by semiflexible frictionless spacer chains of 112Å in length. The "effective" friction bead represents the friction contribution of both the core particle and spacer region, which implies that the spacer region accounts for up to 20% of the friction properties of the dimer (estimated from (R'-R)/R'). Since 62Å was calculated using the minimum reported value of 40 base pairs (12) for the length of DNA in the erythrocyte spacer region, the 20% contribution to the frictional properties of the dimer represents an upper limit. Comparison of f/f_o for several models are presented in Table I for n=12. The random coil calculations were computed from values of σ_{12}, in units of adjacent bead separation, reported by Filson and Bloomfield (21) and employed in the equation,

$$f/f_o = \frac{(12)(74)}{(94.87)(1+(\sigma_{12}/2))} \qquad (8)$$

for contiguous spheres. These values were converted to the "spacer region" model

TABLE I. MODEL COMPARISON OF f/f_o FOR n = 12 BEADS

CONFORMATION	n	f/f_o	
		R = 74Å; B = 148Å	R = 62Å; B = 236Å
Rod (a)	12	3.019	3.8
Regular Polygon (a)	12	2.618	3.4
Contact Helix			
5 elements/turn (a)	12	---	2.397
6 elements/turn (b)	12	1.984	2.480 (c)
5 elements/turn (a)	12	1.925	---
4 elements/turn (b)	12	1.892	2.365 (c)
Random Walk (b)			
Free space	12	2.446	3.057 (c)
Tetrahedral lattice	12	2.459	3.074 (c)
Cubic lattice	12	2.330	2.913 (c)
Experimental (d) ($M = 3.2 \times 10^6$)	12	$2.28 \pm .07$	

(a) present study

(b) calculated from σ_{12} reported by Filson and Bloomfield (21)

(c) estimated from the contiguous sphere model by using the conversion factor $3.8 : 3.0 \simeq 1.25$ (cf. rod calculations).

(d) The value n = 12 is an average number computed from the average molecular weight of the multimer sample and the dimer weight of 5.3×10^5.

by the conversion factor 1.25, obtained as a lower limit to the ratio of rod and regular polygon conformations (cf. Table I).

A salient feature of Table I is the inability of the rod or regular polygon conformations in either the contiguous bead or spacer models to adequately represent the experimental data. It is also clear that the contact helix, defined as a helix in which friction beads in adjacent helical turns touch, is not consistent with the experimental results. The contiguous bead helix can be made to coincide with experiment, however, by increasing the center-to-center distance between adjacent helix turns to a value between 1-1/2 to 2 times the friction bead radius, thereby increasing the exposure to the solvent. The contact helix in the spacer model, which employs a more realistic radius for the friction bead, is in good agreement with all experimental values of f/f_o in the molecular weight range $5 \times 10^5 - 5 \times 10^6$. The parameters used in these calculations are an outside helix radius of 300Å and an angle of inclination of ~4.7°. These do not constitute a unique set of parameters, however, since a decrease in the helical radius, which results in more hydrodynamic shielding, can be compensated for by increasing the angle of inclination. The random walk conformations yield f/f_o values higher than the corresponding experimental values. We cannot rule out the possibility that there are attractive interactions between core particles causing a slight shrinkage of the flexible coil conformation.

The molecular weight of the dimer ($5.3 \pm .25 \times 10^5$) is also consistent with the spacer model (19) as well as the protein composition of the dimer fraction (32). Assuming a core particle weight of 2.10×10^5 and a minimum 80 base pairs of non-core DNA at 660 daltons per pair (the minimum 40 base pairs for the spacer region and 20 base pair "tails" at each end since these are not limit-digest samples (11)), we have a total weight of $\sim 4.73 \times 10^5$. The remaining $\sim 5.7 \times 10^4$ daltons can be partially attributed to the presence of lysine-rich histones in the dimer particle. This 5.7×10^4 dalton difference may be regarded as an upper limit for the lysine-rich histone content since up to an additional 20 base pairs DNA may be in the spacer regions. Nonetheless these calculations support the concept that lysine-

rich histones are associated with dimer particles as postulated (11, 32).

Conclusions

1) The power dependence of the sedimentation coefficient on the molecular weight (a = .554) suggests that the supramolecular structure of the nucleosomes is compact.

2) Model calculations using the Kirkwood theory to analyze the dependence of f/f_o as a function of molecular weight eliminates the rigid rod, regular planar polygon, random walk, and closest-packed spherical structures (since $f/f_o \rightarrow 1$) as possible conformations obtained by polynucleosomes in our samples.

3) The calculations presented herein suggests that, in low ionic strength solutions, either a helical conformation or a flexible-coil polymer with attractive interactions between core particles is obtained by the chromatin multimers.

4) Analysis of the friction factor ratio f/f_o for the dimer suggests that spacer regions are important in interpreting hydrodynamic properties of polynucleosomes containing lysine-rich histones.

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