protein promotes homologous pairing and strand exchange reactions between duplex DNA molecules. *Proc. Natl. Acad. Sci. USA.* 78:6149–6153.
9. Flory, J., S. S. Tsang, and K. Muniyappa. 1984. Isolation and visualization of active presynaptic filaments of recA protein and single-stranded DNA. *Proc. Natl. Acad. Sci. USA.* 81:7026–7030.
10. Di Capua, E., A. Engel, A. Stasiak, and T. Koller. 1982. Characterization of complexes between recA protein and duplex DNA by electron microscopy. *J. Mol. Biol.* 157:87–103.
11. Howard-Flanders, P., S. C. West, J. R. Rusche, and E. H. Egelman, 1984. Molecular mechanisms of general genetic recombination: the DNA binding sites of recA protein. *Cold Spring Harbor Symp. Quant. Biol.* 49:571–580.

CHROMATIN FOLDING INTO HIGHER ORDER STRUCTURE

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An exciting chapter in the study of biological structure is reaching quantitative levels with recently reported advances in nucleosome structure determination. The structure and structural transitions of chromatin, of which the nucleosome is a repeating unit, is presently not amenable to high resolution by x-ray diffraction because of the size of the particles ($\sim 1.2 \times 10^7$ dalton for a chromatin sample comprising 50 nucleosomes with radii of gyration R_g between 30 and 100 nm, depending on experimental conditions). It thus becomes necessary to use lower resolution electron microscopy or solution procedures. Hydrodynamics, sedimentation in the ultracentrifuge and diffusion, and the study of dependence of the intensity of scattered radiation (light, x-rays or neutrons) on the scattering angle, have been methods of choice. In this contribution we would like to demonstrate that conclusions derived from one or two methods only are usually model dependent and do not lead to uniquely defined results. We have therefore combined total intensity and quasielastic light scattering, ultracentrifugation, and small-angle x-ray scattering to characterize chromatin folding with a minimum of model-dependent assumptions. A self-consistent model of chromatin folding into the higher order structure, achieved with increase in either NaCl or $MgCl_2$ concentrations, will be presented. Better characterization of chromatin folding is important in understanding how DNA is packaged in higher organisms into tractable structures, and yet remains easily accessible for biological function.

Chromatin is a complex of double-stranded DNA with the core histones H2A, H2B, H3, and H4 and the linker histones Hl and H5 (in the case of chicken erythrocyte chromatin), organized in chromatosome structure. The repeat length for chicken erythrocyte chromatin, with which we are concerned, is 208 DNA base-pairs, of which 165 are coiled in two superhelical turns in the chromatosome and 43 form the linker connecting the chromatosomes. At low salt concentrations, ~1–2 mM NaCl at neutral pH, chromatin is in a flexible, unfolded so-called "10 nm" filament form. Details of chromatosome arrangement in the structure have been presented (1), though the three-dimensional spatial arrangement is not well understood. With increase in NaCl concentration chromatin folds into a more compact so-called "30 nm" higher order structure. Similar effects can also be achieved by the addition of small quantities of $MgCl_2$. This process has been followed by electron microscopy and x-ray diffraction (2). In solution it has been studied by the determination of frictional coefficients by sedimentation and diffusion (2); shape and size parameters have been studied by light and small-angle x-ray scattering (3, 4) and electrical dichroism (5). Though it is generally believed now that the higher order folded structure is a solenoidal helix, and not an arrangement of superbeads of chromatosomes, its precise nature has not been established. A solenoidal structure with 11 nm helical pitch, ~15 nm radius, and 6–8 chromatosomes per tightly wound helical turn is currently favored.

METHODS

Chromatin from chicken erythrocytes was prepared by digesting nuclei to ~1% acid solubility with micrococcal nuclease in 100 mM KCl, 50 mM Tris-HCl (pH 8.0), 1 mM $CaCl_2$. The reaction was stopped by addition of EDTA to 5 mM and the reaction mixture cooled to ice temperature. Nuclei were lysed and chromatin extracted in various ways (3). Well-characterized chromatin fractions were obtained by sedimentation on a sucrose gradient at 4°C, in 25 mM NaCl, 5 mM Tris-HCl (pH 8.0), 0.1 mM EDTA. The solubility of the chromatin fractions was studied in NaCl, in mixtures of NaCl and $MgCl_2$, and in a variety of other divalent cations (6).

RESULTS

After the NaCl concentration was increased from 1 or 5 mM to 37.5 M NaCl, both the sedimentation coefficient s, determined in the ultracentrifuge, and the diffusion coefficient D, determined by quasielastic light scattering, increased; the radius of gyration R_g, determined by total laser intensity light scattering, decreases correspondingly, indicating a decrease in size of the total structure. At the same time molar masses M, determined by light scattering intensity and by combination of s and D (the Svedberg equation) remain unchanged, in good correspondence with

M calculated from the molar masses M of the DNA isolated from the chromatin fractions. Fractions containing 22–53 chromatosomes per particle (calculated according to the z-average, N_z) were studied (3).

From the values of R_g, and assuming a rigid elongated solenoidal higher order structure for chromatin at 75 mM NaCl (or at 0.3 mM $MgCl_2$ at low chromatin concentrations) it is possible to calculate values L for the lengths of the cylinders, assuming a value $R = 13.1$ nm for the radius. Assuming a pitch $P = 11$ nm, we calculate the number n of chromatosomes per helix turn by

$$n = NP/L \tag{1}$$

to be equal to 5.7, i.e., close to six. This result is in good accord with recent estimates from electrical dichroism (5). Calculation of the frictional parameters by use of the same model dimensions led to values 20% lower than those evaluated from experiment. This, in itself, is not sufficient proof of the inadequacy of the model tested, though it is possible to fit satisfactorily both light scattering and frictional data to an expanded helix with $P = 35$ nm, $R = 23$ nm, and $n = 12$. These dimensions are close to values previously obtained by Fulmer and Bloomfield (7). In continuation, we proceeded to analyze chromatin samples by small-angle x-ray scattering using position-sensitive detectors. Whereas light scattering and frictional parameters probe global size and shape parameters of the chromatin particles, small-angle x-ray scattering is expected to disclose structural features at somewhat higher resolution.

Chromatin samples were analyzed at concentrations between 1 and 5 mg/ml, at NaCl concentrations between 1 and 75 mM NaCl, and in solutions at up to 1.2 mM $MgCl_2$, 40 mM NaCl. Scattering vectors q ranged from 0.15–0.85 nm^{-1}. Analysis was performed according to the cross-section plot, applicable to cylindrical structures. In this representation

$$\ln qI(q) = \ln K(M/L) - 0.5\, R_c^2\, q^2, \tag{2}$$

where K is a constant, M/L is the mass per unit length and R_c is the radius of gyration of the cross-section.

At low concentration of salt, analysis by Eq. 2 correctly yielded $R_c = 2.7$ nm and $M/L = 2 \times 10^4$ d/nm, characteristic of the lower order "10 nm" filament chromatin structure. The striking observation was that up to 40 mM NaCl no change whatsoever in scattering pattern was observed, at ionic concentrations at which considerable folding is monitored by light scattering and frictional properties (the samples analyzed by small-angle x-ray scattering were found to behave properly when tested by light scattering and sedimentation both before and after x-ray irradiation). Only when NaCl concentration was raised above 40 mM, or when combinations of $MgCl_2$ and NaCl were used, did a second higher slope appear in the cross-section plots, with a break near $q = 0.3$ nm^{-1}. Both slope and intercept of this second line relate to the parameters of the higher order structure. Similar plots have been obtained by other workers (8, 9).

Model simulations of x-ray scattering plots favor a model similar to the Fulmer and Bloomfield structure described above, at reasonably low concentrations of salt. The helix turns are spaced such that the x-rays probe the unchanged "local" lower order structures, though overall compaction is monitored by light scattering and examination of frictional properties. Only at the higher concentrations of NaCl and of $MgCl_2$ is a more compact higher-order structure observed. We are thus gaining some insight into the mechanism of chromatin folding into higher-order structures in solution.

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REFERENCES

1. Finch, J. T., and A. Klug. 1976. Solenoidal model for superstructure in chromatin. *Proc. Natl. Acad. Sci. USA.* 73:1897–1901.
2. Butler, P. J. G. 1983. The folding of chromatin. *Crit. Revs. Bioch.* 15:57–91.
3. Ausio, J., N. Borochov, D. Seger, and H. Eisenberg. 1984. Interaction of chromatin with NaCl and $MgCl_2$. *J. Mol. Biol.* 177:373–398.
4. Perez-Grau, L., J. Bordas, and M. H. J. Koch. 1984. Chromatin superstructure: synchroton radiation x-ray scattering study on solutions and gels. *Nucl. Acids Res.* 12:2987–2996.
5. McGhee, J. D., J. M. Nickol, G. Felsenfeld, and D. C. Rau. 1983. Higher order structure of chromatin:orientation of nucleosomes within the 30 nm, chromatin solenoid is independent of species spacer length. *Cell.* 33:831–841.
6. Borochov, N., J. Ausio, and H. Eisenberg. 1984. Interaction and conformational changes of chromatin with divalent ions. *Nucl. Acids Res.* 12:3089–3096.
7. Fulmer, A. W., and V. A. Bloomfield. 1982. Higher order folding of two different classes of chromatin isolated from chicken erythrocyte nuclei. A light scattering study. *Biochemistry.* 21:985–992.
8. Damaschun, H., G. Damaschun, P. A. Pospelov, and V. I. Vorob'ev. 1980. X-ray small angle scattering study of mononucleosomes and of the close packing of nucleosomes in polynucleosomes. *Mol. Biol. Rep.* 6:185–191.
9. Brust, R., and E. Harbers. 1981. Structural investigations on isolated chromatin of higher-order organisation. *Eur. J. Biochem.* 117:609–615.