VISUALIZATION OF CHROMATIN SUBSTRUCTURE: υ BODIES

ADA L. OLINS, R. DOUGLAS CARLSON, and
DONALD E. OLINS

From The University of Tennessee-Oak Ridge Graduate School of Biomedical Sciences, Biology Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee 37830

ABSTRACT

Spread chromatin fibers, from isolated eucaryotic nuclei, reveal linear arrays of spherical particles (υ bodies), about 70 Å in diameter, connected by thin filaments about 15 Å wide. These particles have been observed in freshly isolated nuclei from rat thymus, rat liver, and chicken erythrocytes. In addition, υ bodies can be visualized in preparations of isolated sheared chromatin, and in chromatin reconstructed from dissociating solvent conditions (i.e., high urea-NaCl concentration). As a criterion for perturbation of native chromatin structure low-angle X-ray diffraction patterns were obtained from nuclear pellets at different stages in the preparation of nuclei for electron microscopy. These results suggest that the particulate (υ body) structures observed by electron microscopy may be closely related to the native configuration of chromatin.

The DNA of eucaryotic chromosomes exists in a highly folded condition largely as a consequence of its interactions with the histone proteins (Huberman, 1973; Pardon and Richards, 1973; Hnilica, 1972; DuPraw, 1970). Numerous models of this folded state have been presented based upon data from low-angle X-ray diffraction and from electron microscopy. The models which appear to be favored, at present, consist of superhelical coiling of a fundamental nucleohistone strand (Pardon and Wilkins, 1972; Bram and Ris, 1971). Previous studies from our laboratory (Olins and Olins, 1974) have presented evidence that chromatin fibers consist of chains of spheroid particles about 70 Å in diameter (υ bodies) which could contain DNA folded by association with a small number of histone molecules. This paper extends these observations to soluble chromatin and to reconstructed chromatin. In addition, parallel, low-angle X-ray diffraction studies are included which indicate that the particulate structures observed by electron microscopy are closely related to the native state of chromatin.

MATERIALS AND METHODS

Reagents

Formaldehyde (10%) was prepared fresh by dilution from a stock of 37% analytical reagent grade formaldehyde. Chemicals were reagent grade or better. Buffers and solutions were made from glass-distilled water.

Preparation of Nuclei

Rat thymus, rat liver, and chicken erythrocyte nuclei were prepared as described previously (Olins and Olins, 1974, 1972; Olins and Wright, 1973). Nuclei, recovered after pelleting from high sucrose concentrations, were washed and centrifuged twice in 0.05 M sodium cacodylate buffer (pH 7.5), 0.025 M KCl, 5 mM $MgCl_2$ (CKM), and washed and centrifuged once with 0.2 M KCl before the swelling procedures.

Swelling of the nuclei was generally accomplished by suspending the KCl-washed nuclear pellet in 0.2 M KCl to a concentration of approximately 10^8 nuclei/ml, followed by a dilution of about 200-fold with distilled water. Nuclei were allowed to swell for 10–15 min, then the suspension was made approximately 1% in formaldehyde by the addition of 0.1 vol of 10% formaldehyde (pH

6,8–7.0). Fixation was allowed to proceed for at least 30 min. All operations were conducted at 0°–4°C.

We have recently modified the swelling procedure to minimize clumping of nuclei and improve the spreading of chromatin. Nuclei, after two washes with CKM buffer, are suspended directly in 0.2 M KCl at a concentration of about 10^8 nuclei/ml, and diluted 200-fold with 0.001 M sodium EDTA (pH 7.0) before fixation. This eliminates the centrifugation step in 0.2 M KCl which tends to produce clumping of the nuclei.

Composition of Chicken Erythrocyte Nuclei

The relative histone and nonhistone protein composition for isolated and washed chicken nuclei was measured after extraction of total nuclear proteins with sodium dodecyl sulfate (SDS). After the washing with CKM buffer or the washing with 0.2 M KCl, pellets of nuclei were suspended in 2 ml of 1% SDS buffer (0.01 M sodium phosphate buffer [pH 7.0], 1% SDS, 1% β-mercaptoethanol). After dialysis overnight at room temperature against the 1% SDS buffer, and overnight against 0.1% SDS buffer (0.01 M sodium phosphate buffer [pH 7.0], 0.1% SDS, 0.1% β-mercaptoethanol), the viscous solution was centrifuged for 24 h at 27,000 *g* in a Sorvall RC2-B centrifuge (Ivan Sorval Inc., Newtown, Conn.) at room temperature. The supernate was recovered and examined by electrophoresis in neutral 10% polyacrylamide gels containing SDS, as described previously (Olins and Wright, 1973). Quantitative analysis of stained gels led to the conclusion that nuclei washed with CKM buffer contained 5% nonhistones and 95% histones; washed with 0.2 M KCl, 4% nonhistones and 96% histone proteins. This low content of nonhistone protein for chicken erythrocyte nuclei is consistent with other studies. Saunders (1974) concludes that nonhistone proteins account for $<15\%$ of the total chromosomal proteins. Shelton and Neelin (1971) obtained higher proportions of acid-insoluble proteins, but pointed out that the nuclear preparations contained adhering membranes. It is possible that lysis of erythrocytes with saponin does not adequately remove membrane contamination. We have observed that the chicken erythrocyte nuclei isolated in our laboratory are devoid of membrane material, as assayed by electron microscopy (Olins and Olins, 1972). In addition, Shelton (personal communication) obtains similar low proportions of nonhistone proteins in preparations of chicken erythrocyte nuclei devoid of plasma membrane contamination. Analyses of total RNA content by the method of Savitsky and Stand (1965) and of DNA content by the diphenylamine method (Giles and Myers, 1965) yielded RNA to DNA (wt/wt) ratios of about 0.005:1 for nuclei washed with CKM, or washed with both CKM and 0.2 M KCl.

Preparation of Soluble Chromatin

The method of Zubay and Doty (1959) was slightly modified for the preparation of soluble chicken erythrocyte chromatin. Approximately 200 mg of chicken erythrocyte nuclei, obtained after two washes with CKM buffer, were suspended in 20 ml of saline-EDTA (0.075 M NaCl, 0.024 M sodium EDTA, pH 8.0) plus 0.1 ml *n*-octanol. The nuclear suspension was blended in a VirTis 45 homogenizer (Virtis Co., Inc., Gardiner, N. Y.) for 1 min at one-half full speed and 4 min at one-quarter full speed. The flask was rinsed with 20 ml of saline-EDTA-octanol (of the same composition) and the total homogenate centrifuged for 10 min at 900 *g* in the Sorvall GLC-1. The chromatin pellet was dispersed in 20 ml saline-EDTA plus 0.05 ml *n*-octanol, blended 30 s at one-half full speed, 30 s at one-quarter full speed, and centrifuged again. The pellet was blended in saline-EDTA-octanol (containing 0.05 ml octanol/20 ml saline-EDTA) and recovered by centrifugation, a total of seven times. The resulting chromatin pellet was dispersed rapidly with distilled water by increasing the volume from about 3 to 20 ml while stirring in the VirTis homogenizer at lowest speed. The dispersed chromatin was blended an additional 30 min at lowest speed. Throughout this procedure the chromatin material was kept at 0°–4°C. The chromatin was then dialyzed for 24–48 h against several changes of 0.7 mM sodium phosphate buffer (pH 6.8) at 0°–4°C. After extensive dialysis the chromatin solution was centrifuged for 15 min at 35,000 *g* in a Sorvall RC2-B, and the soluble fraction examined by electron microscopy.

Preparation of Reconstructed Chromatin

Soluble chicken erythrocyte chromatin was dissociated and reconstructed by a step gradient dialysis method (Olins and Olins, 1971) as modified from Bekhor et al. (1969). 1 ml of the soluble chromatin solution (A_{260} ~1–2) was dialyzed overnight, at 0°–4°C, against 1 liter of 3 M NaCl, 5 M urea, 0.001 M sodium cacodylate buffer (pH 7.0). Reconstruction was accomplished by step gradient dialysis (greater than 4 h/step) against solutions of decreasing ionic strength (i.e., 1.0, 0.4, 0.3, 0.1 M NaCl, and 0.0025 M sodium EDTA) in the presence of 5 M urea, with subsequent exhaustive dialysis against 0.001 M sodium cacodylate buffer (pH 7.0) without urea. As a control, 1 ml of "native" soluble chromatin was dialyzed extensively against the 0.001 M cacodylate buffer. Both the native and reconstructed chromatins were examined by electron microscopy.

Preparation of Grids for Electron Microscopy

Spreads of nuclei, of soluble chromatin, and of reconstructed chromatin were prepared by a modification of the procedure of Miller and Beatty (1969 *a*, *b*), as described in our earlier report (Olins and Olins, 1974). Washed and swollen nuclei were diluted to a concentration of about 5×10^5 nuclei per ml in water or in 0.001 M sodium EDTA (pH 7.0), and the suspension was made 1% formaldehyde. Solutions of native or reconstructed chromatin (A_{260} ~0.5–1.5) in 0.001 M sodium EDTA

were made 1% in formaldehyde for at least 30 min. Nuclei and chromatin were centrifuged through 10% formaldehyde (pH 7.0) onto glowed (1 min; maximum voltage; air pressure, 50 μm of mercury), carbon-coated, 400-mesh copper grids. The grids were washed in dilute Kodak Photo-Flo, Eastman Kodak Co., Rochester, N. Y. (3 drops/50 ml water, adjusted to pH 7.0), and air dried. Positive staining with ethanolic phosphotungstic acid, and negative staining with 0.5% ammonium molybdate were described earlier (Olins and Olins, 1974). A solution of 1% phosphotungstic acid (pH 7.1) was also used for negative staining.

For visualization of sonicated fixed nuclei, 1 drop of sonicate was placed on a glowed, carbon-coated grid and removed with bibulous paper. 1 drop of 0.5% ammonium molybdate was placed on the grid and removed by toughing the edge of the grid to the edge of a sheet of bibulous paper. This was repeated twice more, and the grid finally air dried.

Electron Microscopy and Photography

The techniques were the same as described previously (Olins and Olins, 1974, 1972).

Preparation of Samples for X-Ray Diffraction Studies

Aliquots of chicken erythrocyte nuclei were obtained at various steps in the protocol for electron microscopy. Isolated nuclei were washed and centrifuged twice in CKM buffer and an aliquot was removed. A second sample was taken after suspension of the nuclei in 0.2 M KCl. The nuclei were diluted 200-fold into distilled H_2O, allowed to swell 30 min, and a third aliquot was removed. The water-swollen nuclei were fixed with 1% HCHO for 4 h, centrifuged, and resuspended in H_2O to remove excess HCHO. An aliquot of the fixed, water-swollen nuclei was removed. The remaining nuclear preparation was washed with dilute Kodak Photo-Flo, as employed for electron microscopy, and an aliquot taken. Finally, the nuclei were quick-frozen and lyophilized. Samples of the lyophilized nuclei were examined dry and after rewetting with CKM buffer.

The nuclear samples (except for the lyophilized preparations) were pelleted by centrifugation in a Beckman type 65 rotor, at 58,000 rpm for 18 h. A portion of each pellet was pushed into a 1-mm diameter glass capillary (Uni-Mex Company, Griffith, Ind.). A drop of solvent was added at the end of the capillary to prevent the gel from drying, and the capillary closed with sealing wax. The dry lyophilized material was packed dry into the capillary and sealed.

Sample concentrations were measured as described previously (Olins and Olins, 1972), except that the capillaries were crushed in concentrated HNO_3, and analyzed for total phosphate (Amos and Dubin, 1960). The nuclear pellets had a DNA concentration of 70–115 mg DNA/ml, based upon phosphate content and assuming 308 daltons/nucleotide.

X-Ray Diffraction Studies

Low-angle X-ray diffraction data were collected using a Searle X-ray camera with Elliott toroid optics (Baird & Tatlock Ltd., England) and sector apertures. The camera was mounted on an Elliott rotating copper anode X-ray generator (type GX6), operating at 40 kV with a tube current of 40 mA. Data were collected on Kodak type T industrial X-ray film. Three sheets of film were held perpendicular to the beam in a flat film holder. Exposures were carried out for 2 h in air, and the sample-to-film distance was 7.3 cm. The position of reflections was measured as described previously (Olins and Olins, 1972).

RESULTS

Electron Microscope Studies

Isolated eucaryotic nuclei undergo rapid morphological changes in response to alteration of solvent pH and electrolyte concentrations (Olins and Olins, 1972; Brasch et al., 1971; Philpot and Stanier, 1956; Anderson and Wilbur, 1952; Ris and Mirsky, 1949). When they examined their preparations by phase microscopy, these authors observed a progressive disappearance of chromatin condensations and an increase in nuclear volume as nuclei are washed with buffers devoid of divalent cations and with lowered ionic strengths. Evidence has been presented (Olins and Olins, 1972) indicating that this loss of chromatin condensation can occur without disruption of the chromatin periodic folding (i.e., without total disappearance of the characteristic low-angle X-ray reflections). In the studies of Olins and Olins (1972), 0.02 M KCl was employed to obtain swollen nuclei. Brasch et al. (1971) had indicated that additional swelling could be obtained by washing nuclei in distilled water. We have utilized this additional swelling in water as a first step in a procedure to resolve substructure in separate chromatin fibers.

Maximal swelling of isolated nuclei appears to depend upon a judicious choice of solvent washes. Dilution of aliquots of nuclear suspensions from 5 mM $MgCl_2$-containing buffer (i.e., CKM buffer) directly into large volumes of distilled water does not yield complete chromatin dispersion and nuclear swelling. More swelling is obtained by washing the nuclei in a solvent of moderate ionic strength not containing divalent cations (i.e., 0.2

M KCl) followed by dilution into water or low ionic strength buffer.

SPREADING OF WATER SWOLLEN NUCLEI: The method of centrifuging chromosomes onto an electron microscope grid followed by air drying out of Photo-Flo, developed by Miller and co-workers (Miller and Beatty, 1969 *a*, *b*; Miller et al., 1970), has the advantage of spreading and separating the chromatin fibers. Using this method on a variety of isolated eucaryotic nuclei, we have been able to resolve a periodic substructure in chromatin fibers.

Isolated nuclei, washed twice with CKM buffer, once with 0.2 M KCl, diluted into distilled water, and fixed with formaldehyde, were centrifuged onto carbon-coated grids and air dried from Photo-Flo. Chromatin fibers can be observed coming out of the main body of the nucleus. Repeated observations of high magnification electron micrographs, treated with positive stains, suggested the existence of a periodic substructure. The use of negative-staining methods (i.e., ammonium molybdate or phosphotungstic acid) yielded far more convincing evidence (Fig. 1).

Micrographs of negatively stained rat thymus, rat liver, and chicken erythrocyte nuclei revealed the presence of linear arrays of spherical particles (denoted υ bodies) connected by very thin filaments. Measurements of diameters of υ bodies for the different tissues yielded the following average diameters and standard deviations: rat thymus, 83 ± 23 Å; rat liver, 60 ± 16 Å; and chicken erythrocyte, 63 ± 19 Å. Connecting strands (rat thymus nuclei) exhibited average widths of 15 ± 4 Å. Clusters of several υ bodies close packed along chromatin fibers can be seen in Fig. 1. Data has been presented (Olins and Olins, 1974) comparing widths of positively stained chromatin fibers with diameters of υ bodies from negatively stained preparations. It appeared from this comparison that clusters of several υ bodies are not resolved by positive-staining techniques, but appear as chromatin thickenings with fiber widths at 100–125 and 225–240 Å.

SPREADING OF NATIVE AND RECONSTRUCTED CHROMATIN: Soluble nucleohistone material was isolated from purified chicken erythrocyte nuclei by a modification of the techniques of Zubay and Doty (1959). An aliquot of the soluble chromatin was exposed to dissociating conditions (i.e., 5 M urea, 3 M NaCl) by dialysis overnight at 4°C. The dissociated chromatin was reassociated by step gradient dialysis (see Materials and Methods) down to 0.001 M sodium cacodylate buffer, pH 7.0. As a control, an aliquot of native soluble chromatin was dialyzed against 0.001 M sodium cacodylate buffer. Both samples, native and reconstructed nucleohistones, exhibited υ bodies throughout the chromatin fibers (Fig. 2 *a*, *b*). Measurements of the average particle diameters yielded the following data: native chromatin, 54 ± 10 Å; reconstructed chromatin, 53 ± 7 Å.

FRAGMENTATION OF FIXED CHROMATIN BY SONICATION: As a first step to isolate and characterize chromatin fragments resembling υ bodies, water-swollen and formaldehyde-fixed chicken erythrocyte nuclei were exposed to extensive sonication. After treatment with a Biosonik II sonicator (VWR Industries, Rochester, N. Y.) with standard 0.5-inch titanium tip at maximum intensity for 6 min, electron microscope examination of the unfractionated sonicate (Fig. 2 *c*) demonstrated a complex mixture of broken chromatin fragments, the smallest sizes resembling individual υ bodies, and larger fragments containing clusters of υ bodies. This sonicated material can be fractionated into a continuous distribution of size classes by sedimentation in sucrose gradients, and fractions enriched in υ bodies, as determined by electron microscope observations, can be selected for further study (Senior et al., 1975).

X-Ray Diffraction Studies

Isolated hydrated nucleohistone, nuclei, and chromosomes exhibit a series of low-angle X-ray diffraction maxima at about 110, 55, 35, 27, 22, and 18 Å (Wilkins et al., 1959; Luzzati and Nicolaieff, 1959, 1963; Pardon et al., 1967; Pardon and Wilkins, 1972; Olins and Olins, 1972; Pardon and Richards, 1973). These X-ray reflections are generally regarded as characteristic of native chromatin periodic structure, and furnish a useful criterion for possible perturbations in chromatin structure.

In an effort to determine whether the steps involved in visualizing chromatin by electron microscopy might introduce structural alterations, isolated chicken erythrocyte nuclei were examined by low-angle X-ray diffraction at different stages in the protocol for electron microscopy. Sample preparation techniques are described in Materials and Methods. Table I indicates the results obtained from the nuclear pellets at different stages in the protocol. Fig. 2 *d*,*e* illustrates representative low-angle X-ray diffraction patterns. These data indicate that isolated nuclei processed by washing,

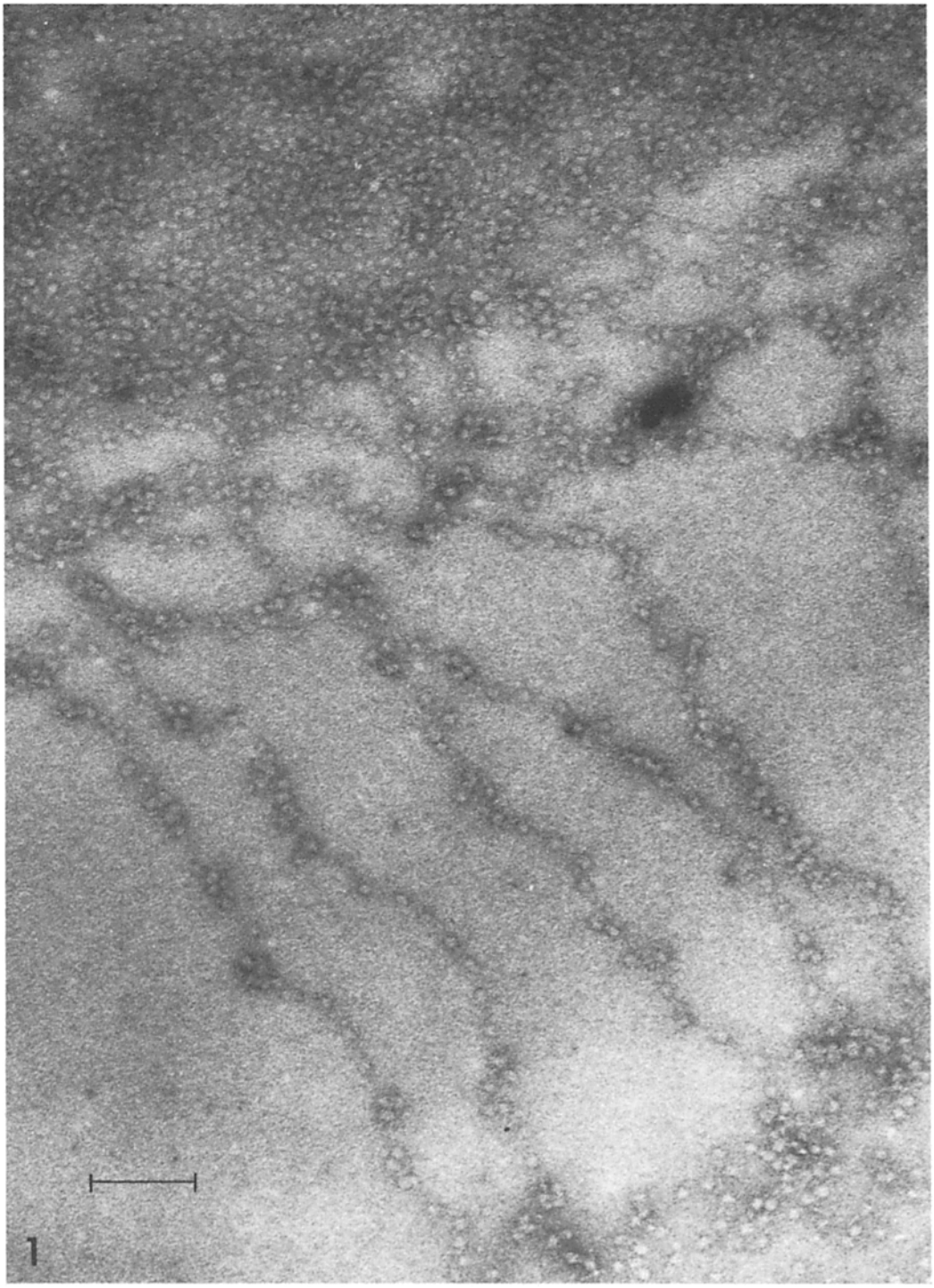

FIGURE 1 Electron micrograph of the edge of a rat thymus nucleus centrifuged onto a carbon-coated grid. A few chromatin fibers are stretched out from a region of higher chromatin concentration. Negative stain with 0.5% ammonium molybdate (pH 7.8). Bar denotes 0.1 μm. × 163,000.

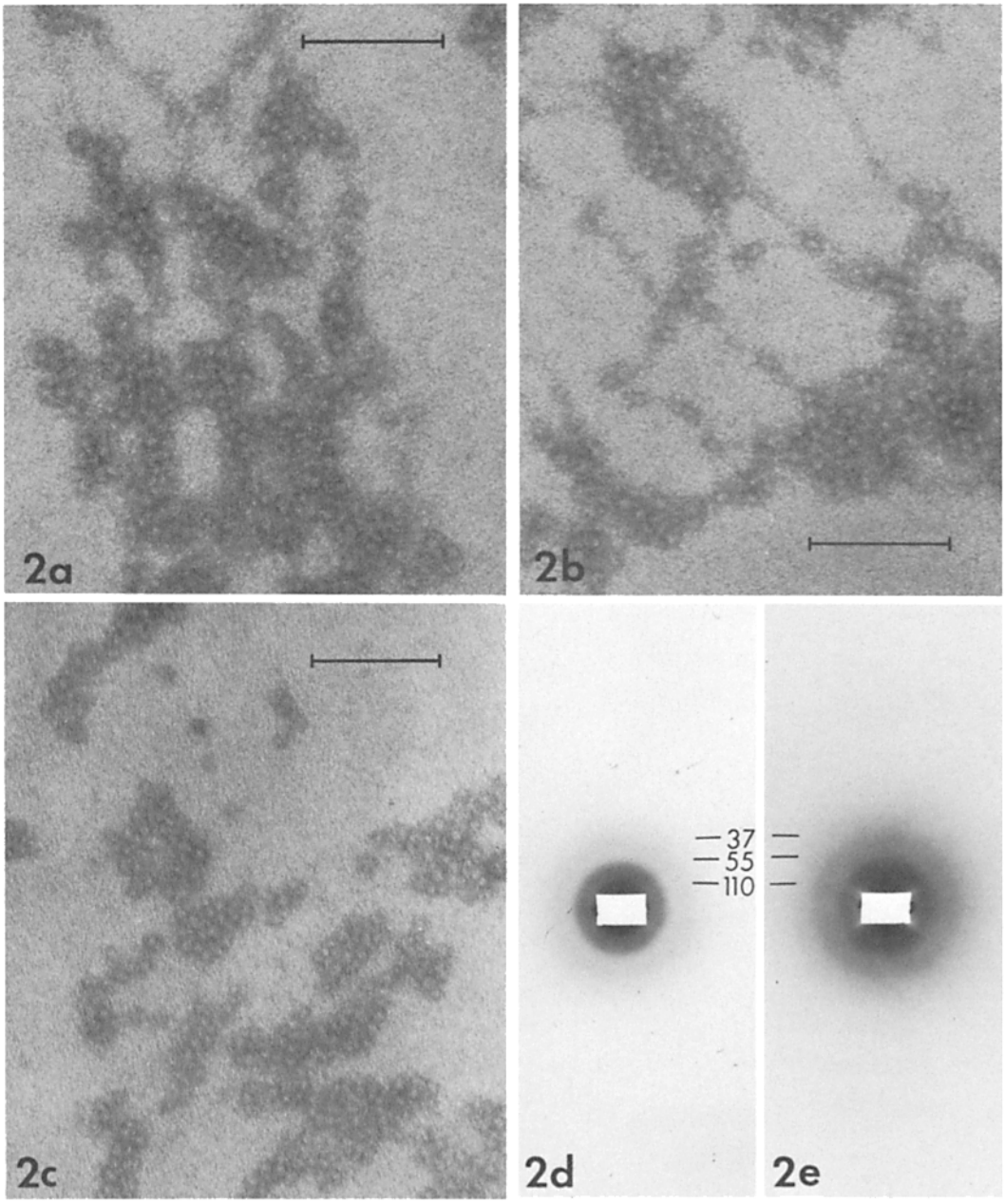

FIGURE 2 (*a*) Soluble chicken erythrocyte chromatin. Negative stain with 1% phosphotungstic acid (pH 7.1). Bar denotes 0.1 μm. × 192,000. (*b*) Reconstructed chicken erythrocyte chromatin. Negative stain with 1% phosphotungstic acid (pH 7.1). Bar denotes 0.1 μm. × 192,000. (*c*) Sonicated chicken erythrocyte nuclei. Negative stain with 0.5% ammonium molybdate (pH 7.3). Bar denotes 0.1 μm. × 177,000. (*d*, *e*) Low-angle X-ray diffraction patterns from nuclear pellets: (*d*) washed in CKM buffer (DNA concentration, 11%); (*e*) rewetted from fixed lyophilized nuclei (DNA, 7%). It has previously been observed that the 110- and 55-Å reflections are not distinct rings for gels in this concentration range (Wilkins et al., 1959, Plate Ia; Pooley et al., 1974). They appear instead as shoulders or edges. Diffraction patterns from more concentrated gels obtained by rewetting dry fibers indeed show these as distinct maxima (Pooley et al., 1974). The use of nuclei has constrained us to the lower concentration range.

TABLE I

Results Obtained from the Nuclear Pellets at Different Stages in the Protocol

Stage of nuclear pellet	Low-angle X-ray reflections*
CKM wash	105, 57, 37
KCl wash	107, 58, 37
H_2O swollen	105, 58, 38
HCHO fixed	107, 58, 37
Photo-Flo wash	102, 58, 38
Lyophilized	(105, 57, 36)‡
CKM rewetted	115, 56, 37

* The reflections at ~110 and 55 Å appear as shoulders. The precision of the positions is about ±10, ±3, ±2 Å, respectively, for the ~110, 55, and 37 Å reflections. Faint reflections at 28 ± 2 and 21 ± 2 Å were also observed on the most exposed films of the nuclear pellet in CKM.

‡ Very faint, but consistently observed in several separate experiments.

swelling, fixation, and treatment with Photo-Flo each exhibit the characteristic low-angle X-ray diffraction pattern of native chromatin. For each of these samples, the ~110-, 55-, and 37-Å reflections can be seen. There is no shift in their positions. The reflections observed from the samples in low ionic strength solvents were of weaker intensity than from the samples in CKM or 0.2 M KCl buffers. Under these low ionic strength conditions the nuclear pellets did not compact as much by ultracentrifugation as those at the higher ionic strengths. In separate experiments, the swollen, fixed, and Photo-Flo samples were made to higher ionic strength by the addition of equal volumes of double concentrated CKM buffer, before ultracentrifugation. The intensities of the diffraction patterns were then more similar to those of CKM-washed nuclei. The fixed, lyophilized erythrocyte nuclei exhibited very weak reflections at the positions of native chromatin. This contrast with results from unfixed and dried chromatin (Carlson and Olins, 1974; Pooley et al., 1974) where X-ray reflections were observed at ~76 and 38 Å, and suggests that fixation of chromatin with formaldehyde tends to stabilize the native structure against the disruptive effects of drying. Rehydration of lyophilized chromatin (fixed or unfixed) resulted in restoration of the native X-ray diffraction reflections. No attempts were made to examine the X-ray diffraction patterns of nuclear material dispersed within the electron-dense stains employed for microscope studies. In a study on the effects of varying times of fixation, treatment of chicken erythrocyte nuclei with 1% HCHO for up to 24 h, yielded no significant alteration of the X-ray pattern. It is possible, however, that formaldehyde fixation of native chromatin does lead to some perturbation of the DNA structure. Senior and Olins (1974) have observed that formaldehyde enhances the "C"-type circular dichroic spectrum of soluble erythrocyte chromatin. It has also been shown that formaldehyde fixation renders the histones nonextractable by acid, high salt, guanidine hydrochloride, and SDS (Senior and Olins, 1974; Brutlag et al., 1969), and markedly perturbs the thermal denaturation profile of chromatin (Li, 1972; Brutlag et al., 1969).

DISCUSSION

The present study demonstrates that chromatin fibers can be visualized to possess a periodic substructure; i.e., linear chains of spheroid particles (υ bodies), approximately 70 Å in diameter, connected by thin filaments about 15 Å wide. We believe that υ bodies represent, at least, a vestige of a periodic substructure of native chromatin. The evidence favoring the view that native chromatin possesses a particulate substructure includes the following observations. (*a*) Low-angle X-ray scattering studies of isolated nuclei processed by the protocol employed for electron microscopy exhibited minimal perturbations up to the drying stage. If the spheroid particles (υ bodies) observed by electron microscopy represent perturbations of the chromatin substructure, dehydration of the chromatin appears to be a likely cause. The effects of dehydration, however, appear to be largely reversed by rewetting the chromatin material. (*b*) Extensive sonication of fixed chromatin produces fragments resembling monomer υ bodies. Fractionated or unfractionated sonicates exhibit the chromatin particles in the electron microscope. Drying out of Photo-Flo is not necessary to visualize the υ bodies (see Materials and Methods). These monomer υ bodies have been isolated by sucrose gradient ultracentrifugation, and characterized in terms of particle molecular weight (~300,000 daltons/υ body), protein to DNA ratio (1.22/1), and DNA fragment molecular weight (~140,000 daltons of DNA/υ body) (Senior et al., 1975). (*c*) Spheroid nucleohistone particles have been produced by treatment of unfixed chromatin with micrococcal nuclease (Rill and Van Holde, 1973; Sahasrabuddhe and Van Holde, 1974). These particles are about half the molecular weight

of the monomer υ bodies obtained from fixed material. The basis of this difference remains to be elucidated. The evidence suggests, nonetheless, that both unfixed and formaldehyde-fixed chromatin possess periodic "weak" points with enhanced susceptibility to breakage by nuclease or by sonication.

It is important to ask why other methods of visualizing chromatin ultrastructure have not indicated a particulate structure similar to the υ bodies. One prominent method (Gall, 1966; DuPraw, 1966; Ris and Kubai, 1970; Bram and Ris, 1971; Lampert, 1971) involves spreading of chromatin fibers upon an aqueous hypophase, picking up the fibers on a microscope grid, fixation with formaldehyde, dehydration through a graded series of ethanol concentrations, transfer to amyl acetate, and drying by the critical-point method (Anderson, 1951). Employing this method, chromatin generally appears as an irregularly folded thread with thickness varying between 100 and 250 Å depending upon the presence of divalent ions during the spreading procedure. We have examined many of the published micrographs employing this procedure and have never observed large regions with structures resembling the υ bodies. Simultaneously with our description (Olins and Olins, 1973) of υ bodies in chromatin preparations, Woodcock (1973) reported observing similar particles in spreads of osmotically lysed amphibian and avian erythrocytes. He pointed out that the particles were not observable when the chromatin was dried from ethanol or amyl acetate. Pooley et al. (1974) have examined the influence of drying from ethanol or amyl acetate upon the low-angle X-ray reflections. Preparations dried from ethanol or amyl acetate did not show the characteristic dry pattern (i.e., 76 and 38 Å), and did not readily exhibit the low-angle reflections of native chromatin when rewetted. These authors conclude that "drying (of chromatin) from water retains some order; dehydration from organic solvents, either by vacuum evaporation or the critical-point method, retains less order" (Pooley et al., 1974). We suggest that the reason why previous investigators have not observed the υ bodies is that drying chromatin from nonaqueous solvents produces drastic structural perturbations.

Slayter et al. (1972) have examined the ultrastructure of soluble, unfixed chromatin preparations sprayed through a high-pressure spray gun onto microscope grids, vacuum dried from ammonium acetate buffers of varying ionic strength, and shadow cast with evaporated platinum. These authors do observe some spherical particles ("nodular elements") about 150 Å in diameter attached to strands 20–30 Å wide. These structures may correspond to the υ bodies. Their chromatin exhibits considerable branching, aggregations, and many long stretches devoid of nodular elements. It is possible that chromatin receives considerable shear stretching during the spraying procedure. Pardon et al. (1967) have observed that the periodic structure of chromatin (i.e., as observed by low-angle X-ray diffraction) is destroyed by excessive stretch. It is conceivable that the spraying of unfixed chromatin results in a localized loss of the spheroid chromatin units.

A similar argument (i.e., the sensitivity of chromatin to stretch) makes it difficult for us to interpret the strands connecting υ bodies, in our micrographs. They might represent regions of "naked" (or "accessible") DNA within the chromatin (see Huberman, 1973). However, they might also represent localized stretching and unravelling of the υ bodies. One possible model for the υ body, currently being explored in our laboratory, assumes that at least a portion of the chromatin DNA forms a "shell" around the particle, which is accessible to nuclease, dyes, and polylysine, and can be unravelled from the particle by stretching of the chromatin.

It is likely, but not proven, that υ bodies are visualized in an extensively dehydrated state. It is worth noting that the volumes of ribosomal particles and subunits calculated from electron microscope observations are roughly half of the calculated volumes from hydrodynamic measurements (Hill et al., 1969). If such a volume shrinkage due to dehydration also occurs with the υ bodies, the diameter of these particles would be increased from approximately 70 Å (dehydrated) to approximately 90 Å (hydrated).

Assuming the existence of υ body-like structures within the native unfixed chromatin, one can ask how such a particle structure might relate to the characteristic low-angle X-ray reflections observed with chromatin (Pardon et al., 1967; Pardon and Wilkins, 1972). There would appear to be at least two classes of possible relationships. In the first class, the X-ray reflections would arise solely from the packing of chromatin particles (i.e., the diffraction pattern would be due to the interparticle spacings in the packing lattice). This class of structures would predict that, if isolated monomer υ bodies exhibit the low-angle X-ray reflections,

dilution of the particles should alter the diffraction positions (i.e., the diffraction spacings would be concentration dependent). In the second class, the X-ray reflections would arise solely from the intraparticle structure. This would predict that the observed diffraction positions would not be altered by dilution of isolated monomer υ bodies (i.e., the diffraction spacings would *not* be concentration dependent). Additionally, some of the reflections could arise from the particle packing lattice, others from intraparticle structure. Preliminary observations (Carlson and Olins, unpublished) have shown that chromatin fractions enriched with monomer υ bodies exhibit the characteristic low-angle X-ray reflections; i.e., the periodic structure has survived fixation, sonication, and fractionation of the chromatin. A concentration-dependence study is currently in progress.

The authors gratefully acknowledge the advice and criticism of Doctors Marilyn B. Senior and Ralph Einstein. We also wish to express our appreciation to Everline B. Wright and Mayphoon Hsie for excellent technical assistance.

This work was supported, in part, by the U. S. Atomic Energy Commission under contract with Union Carbide Corporation, and in part by a research grant to Dr. D. E. Olins from the Public Health Service (GM 19334). Dr. R. D. Carlson is a recipient of a National Institutes of Health postdoctoral fellowship (GM 55427).

Received for publication 25 June 1974, and in revised form 18 November 1974.

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