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Chromatinν bodies: isolation, subfractionation and physical characterization

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ABSTRACT

Monomer chromatin subunit particles (ν_1) have been isolated in gram quantities by large-scale zonal ultracentrifugation of micrococcal nuclease digests of chicken erythrocyte nuclei. ν_1 can be stored, apparently indefinitely, frozen in 0.2 mM EDTA (pH 7.0) at ≤ -25°C. Aliquots of the stored monomers have been subfractionated by dialysis against 0.1 M KCl buffers into a soluble fraction containing equimolar amounts of H4, H3, H2A, H2B associated with a DNA fragment of ~130–140 nucleotide pairs, and a precipitated fraction containing all of the histones including H5 and H1 associated with DNA fragments. The total ν_1 and the KCl-soluble fraction of ν_1 have been examined by sedimentation, diffusion, sedimentation equilibrium ultracentrifugation, low-angle X-ray diffraction, and electron microscopy. Physical parameters from all of these techniques are presented and correlated in this study.

INTRODUCTION

There is now considerable evidence that the nucleohistone component of eukaryotic chromosomes is organized into a string of globular elements (the ν bodies or nucleosomes) joined by nuclease-sensitive "connecting strands." The veritable flood of consistent data since 1973 includes evidence from: electron microscopy (1–17), nuclease digestion (18–41), histone-histone interactions and chemical cross-linking (42–57), X-ray and neutron scattering (4, 47, 48–62), and estimates of histone stoichiometry (3, 63–65). Measurements of the DNA length per ν body have been complicated by tissue variation, the extent of digestion, and variations in measurement technique and calibration standards (21, 25, 28, 30, 32, 35). Clearly, extensive physical characterization of the nuclease-derived monomer ν bodies (ν_1) requires isolation under conditions that maximize yields and minimize variations in the extent of digestion. The present study represents the beginnings of such an extensive study on the properties of ν_1 obtained from chicken erythrocyte nuclei.

METHODS

Small-Scale Nuclear Isolation for the Study of Digestion Kinetics

Chicken blood (5 ml) was collected in 30 ml of buffer containing 10 mM NaCl, 10 mM Tris-HCl, and 3 mM $MgCl_2$ (pH 7.4) (STM buffer). Cells were pelleted at low speed (10 min, 4000 X g) and lysed at 4° in STM buffer plus 0.5% Nonidet P40 (Particle Data Lab Ltd., Elmhurst, Ill.) (STMN buffer). Nuclei were washed 3 times and resuspended in STMN buffer to a concentration of 1.4×10^9 nuclei/ml. The solution was made 10^{-3} M $CaCl_2$, 10^{-3} M phenylmethylsulfonyl fluoride (PMSF), 0.2% isopropanol (the solvent for PMSF), 6 µg/ml micrococcal nuclease (Worthington Biochemical Corp.), and the samples were incubated at 37° for various lengths of time. Digestion was terminated by the addition of ice-cold EDTA (pH 7.0) to 40 mM, followed by centrifugation at low speed. The nuclear pellets were dispersed in 0.2 mM EDTA (pH 7.0) (final $A_{260} \sim 50$–100). Release of acid-soluble nucleotides was measured as the fraction of DNA soluble in 0.4 M perchloric acid–0.4 M NaCl (30).

Large-Scale Isolation of ν_1

For the very large-scale production of ν_1, eight chickens were exsanguinated, and their blood (540 ml) was collected into 60 ml of anticoagulant citrate dextrose (Aminco, Silver Springs, Md.) to prevent clotting. The blood was filtered through gauze, diluted with 300 ml STM buffer, and centrifuged for 15 min at 4000 X g. The loose erythrocyte pellet was suspended in 1.5 liters of STMN buffer, stirred with a magnetic bar for 10 min, and centrifuged; the entire washing cycle was repeated three more times. The resulting nuclear preparation was diluted to a concentration of 1.4×10^9 nuclei/ml (860 ml), made 10^{-3} M $CaCl_2$, 1 mM PSMF, 2% isopropanol, and 6 µg/ml micrococcal nuclease. The material was digested for 6 1/2 hr at 37°, with occasional shaking. The reaction was terminated by addition of 174 ml of 200 mM EDTA and rapid chilling. After centrifugation (15 min, 6000 X g), the digested nuclear pellet was resuspended in 120 ml of 0.2 mM EDTA, 1 mM PMSF, 2% isopropanol; stirred; and brought to a final concentration of ~10 mg chromatin/ml (i.e., $A_{260} \sim 100$).

Conditions and results of fractionation in the K-VIII zonal ultracentrifuge have recently been described elsewhere (65). In this manner we have fractionated a single digest containing ~4.5 g of erythrocyte chromatin in three separate zonal centrifuge runs, yielding a total of ~1.5 g of ν_1. We have subsequently determined that centri-

fugation of the total digest for 1.5 hr at 2000 X g in an International PR-2 refrigerated centrifuge to remove the high-molecular-weight chromatin allows fractionation of the remaining soluble chromatin digest in a single K-VIII zonal run.

The peak fraction was concentrated by precipitation with 10 mM $MgCl_2$, 1 mM PMSF, 2% isopropanol, overnight at ~4°. The supernatant was gently removed by suction, and the pellet was further concentrated by centrifugation. The loose pellet (~50 ml) was subsequently dialyzed against 5 liters each of 20 mM, 2 mM, and 0.2 mM EDTA. The final product was an almost clear solution of monomer particles (~60 ml of A_{260} ~100–150).

The solution of ν_1 was dispersed into 0.5-ml aliquots and stored frozen in screw-cap vials at -25°. Hydrodynamic and electron microscopic evidence suggests that a single vial can be repeatedly frozen and thawed with minimal physical consequences. For the present study, however, small aliquots were thawed, dialyzed against the appropriate buffer, and analyzed as soon as possible.

Analytical Fractionation Techniques

Sucrose gradient ultracentrifugation was performed as described previously (5, 65) in a 5–20% linear sucrose gradient, containing 0.2 mM EDTA.

DNA, from digested nuclei or ν_1, was purified by dispersion of the chromatin in EDTA–$NaClO_4$–SDS (sodium dodecyl sulfate), followed by phenol, and chloroform-isoamyl alcohol extraction [techniques modified from previous authors (66–68)]. The ethanol-precipitated DNA was dissolved in 0.2 mM EDTA (pH 7.0) at A_{260} ~50–80.

DNA fragments were electrophoresed in 5% polyacrylamide gels polymerized in glass tubes (9 cm X 0.5 cm ID) by use of a Tris-borate-EDTA buffer system (69, 70). Aliquots of DNA (15 λ; A_{260} = 20) were electrophoresed with constant current regulation. Gels were stained with toluidine blue, destained by diffusion in water, photographed, and scanned at 546 mμ (71) in a Gilford Model 2000 automatic recording spectrophotometer. Mobility profiles of the DNA fragments were compared with a total digest of chicken nuclei prepared, calibrated, and provided by B. Sollner-Webb and G. Felsenfeld (30).

Electrophoresis of ν_1 fractions and of total chromatin fragments from nuclease digestion, was also performed in 5% polyacrylamide disc gels (9.0 cm X 0.5 cm ID) with all buffers reduced in concentration by a factor of 10, compared with the DNA gels (30). Gels were stained with toluidine blue. Sample loads were 15–30 λ of a solution

with A_{260} ~20.

Histone content and relative molar ratios were determined after electrophoresis of SDS-dissociated chromatin fragments on 18% polyacrylamide gels (pH 8.8) containing 0.1% SDS (48, 65, 72). Gels were stained with 0.1% amido black, destained slowly in the presence of free dye, scanned, and compared with calibrated histone standards as previously described (64, 65). We have occasionally employed Coomassie blue as a protein stain for the analysis of SDS gels. However, the technique of slow equilibration of the gels against free dye does not yield linear standard curves for the purified histone standards. It also appears that weakly stained protein bands destain more rapidly and completely than the stronger-staining bands. We have, therefore, continued to employ amido black even though it is regarded as less sensitive than Coomassie blue. Our views on the pitfalls of the analysis of acid-extracted histones in terms of relative molar ratios, which are discussed in more detail elsewhere (65), justify our emphasis on the analysis of histones extracted by SDS.

Two-dimensional gel electrophoretic analyses of the total ν bodies and of the 0.1 M KCl-soluble fraction consisted of a first-dimension electrophoresis of the chromatin particles in 5% polyacrylamide gels, as described above, but with sample load increased to 50 λ of a solution with $A_{260} = 80$. The unstained gels were incubated in the SDS sample buffer for 1–2 hr at 37°, and subsequently sandwiched between two glass plates taped and clamped into a slab shape (10 cm long X 15.5 cm wide X 0.25 cm thick). An 18% SDS-containing separation gel was polymerized underneath the disc gel, and a 6% SDS-containing stacking gel was polymerized around it. Electrophoresis was performed at ~50 ma with current regulation until the marker bromophenyl blue dye had reached the bottom (~17 hr). Gel slabs were stained with amido black, destained, and scanned as described above.

Hydrodynamic Techniques

All ultracentrifugal analyses were performed in a Model E analytical ultracentrifuge equipped with UV optics, scanner, and multiplexer units. Samples were dialyzed extensively against their respective buffers prior to ultracentrifugal analyses. Data were analyzed on an Olivetti Programma 101 calculator employing programs provided by R. Trautman (73). Scanner data for all runs were recorded at 265 nm.

Sedimentation coefficients were measured by boundary sedimentation over the concentration range $A_{260} = 0.20–1.00$, at 24,000 rpm and 25° in an An-G rotor.

Sucrose (0.5%) was added to half of each solution to stabilize against mechanical and thermal mixing.

Diffusion coefficients were determined by boundary-spreading analysis of pure solvent layered over solutions of chromatin particles (A_{260} = 0.20–1.00) in buffer plus 0.5% sucrose to stabilize the synthetic boundary. Diffusion experiments were performed at 4800 rpm and 25°.

Particle and DNA molecular weights were determined by equilibrium sedimentation ultracentrifugation in 3-mm columns over a fluorocarbon layer at speeds adequate to yield meniscus-depletion of solute. These speeds had the additional advantage of stabilizing the concentration gradient against mixing. The temperature control unit (RTIC) was turned off during the sedimentation equilibrium runs.

Conversion of the sedimentation coefficient (S) and the diffusion coefficient (D) to standard conditions employed data from various sources (74, 75) as well as a direct measurement of buffer density. The apparent partial specific volume (φ) of the total ν_1 was determined in 10 mM KCl, 0.2 mM EDTA, 0.1 mM PMSF, and 0.2% isopropanol (pH 7.0). Since there was no significant dependence of φ on concentration (five samples analyzed were from 0.5 to 5.5 mg/ml), it was assumed that the value for φ is the partial specific volume ($\bar{v}$). The densities of the five solutions and the dialysate were determined with a Paar Precision Density Meter DMA 02C to a precision of 5×10^6 g/ml. Solutions were prepared by making weight-weight dilutions of a stock solution. The concentration of the stock was determined from dry-weight measurements on a Mettler H20T semimicrobalance following lyophilization and drying of the stock for 2 days at ~95°, in vacuo. These measurements yielded a value of $\bar{v} = 0.661 \pm 0.006$ ml/g. In addition, UV absorption measurements on a Cary 15 spectrophotometer yielded an extinction coefficient for ν_1 of $E^{1\%}_{260\,nm} = 93.12 \pm 0.52$, in the 10 mM KCl buffer.

Small-Angle X-Ray Scattering

The X-ray scattering by pellets of ν_1 was recorded on film using a Searle X-ray camera with Franks optics. 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. The pellets were obtained by centrifuging ν_1 in the 10 mM KCl buffer and in 5.0 mM $MgCl_2$ for up to 40 hr (48,000 rpm in a Beckman model SW50 L rotor, 5°). Dried samples were obtained by opening the special sample holder and placing it in a vacuum for several days.

Electron Microscopy

A drop of a solution containing total ν_1 or 0.1 M KCl-soluble ν_1 was placed on a freshly glowed carbon film for 30 sec, washed in dilute Kodak Photo-flo (pH 7.0), dried, and negatively stained with 0.01 M uranyl acetate (4, 5, 65). Electron micrographs were taken on a Siemens 102, at 80 kv, with the objective lens current between 1209 and 1216 ma. For all micrographs (except ν_1 fixed in formaldehyde), the intermediate lens was normalized after changes in magnification to avoid hysteresis. Four different catalase crystals were photographed at each magnification before and after each sample of ν_1 was photographed. All negatives were printed together at a 3X enlargement. Measurements were made on photographic prints using a Bausch and Lomb 7X magnifier with a graticule divided into 0.005-in. spaces. Only clearly delineated particles were measured on micrographs which were at, or slightly under, focus. We have employed 86 ± 2 Å for the catalase repeat distance in accordance with Wrigley (76).

RESULTS

Kinetics of Digestion with Micrococcal Nuclease

The results of our kinetic analyses resemble quite closely to those previously published by Sollner-Webb and Felsenfeld (30), and it is worthwhile to compare their Fig. 1 with our Fig. 1. We have employed digestion conditions consisting of high substrate

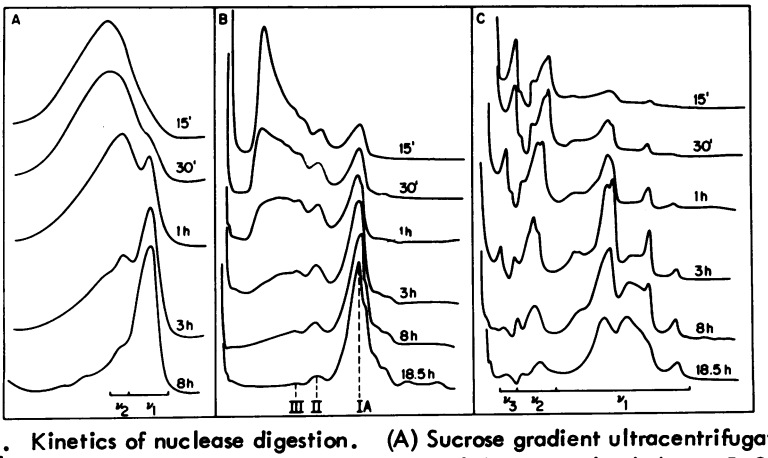

Figure 1. Kinetics of nuclease digestion. (A) Sucrose gradient ultracentrifugation. Sedimentation is from right to left. 0.1 ml of total digest was loaded on a 5–20% sucrose gradient. Centrifugation was for 12 hr at 4° and 35,000 rpm in an SW41 rotor. (B) Electrophoresis of DNA fragments. Migration is from left to right. Fragment notation follows the suggestion of Sollner-Webb and Felsenfeld (30): IA, ~140 np; II, ~370 np; III, ~580 np. (C) Electrophoresis of chromatin fragments. Migration is from left to right. The presumptive regions of ν_1, ν_2, and ν_3 are indicated.

and low enzyme concentration in order to minimize the expense of large-scale preparations of ν_1. In our study, the kinetics were examined in terms of perchloric acid-soluble nucleotides, sucrose gradient profiles, and DNA-fragment and chromatin-fragment gel electrophoresis. Measurements of the percentage of acid-soluble nucleotides for different digestion times were as follows: 15 min, 1.5%; 30 min, 2.5%; 1 hr, 3.5%; 3 hr, 9.5%; 8 hr, 21.5%; 18-1/2 hr, 21.5%. We have no explanation for the apparent plateau at ~22% acid solubility. No other experiments were done with such long digestion times. It is worth mentioning here that our large-scale digests were performed for 6-1/2 hr, which would correspond to ~17–18% acid-solubility. Figures 1A and 1B demonstrate the conversion of multimers (ν_n) to monomers (ν_1), dimers (ν_2), and trimers (ν_3) as a function of time of digestion. Each fraction has been identified by electron microscopy (64). DNA fragments are referred to as IA, II, and III following the nomenclature of Sollner-Webb and Felsenfeld (30).

Electrophoretic analysis of the chromatin particles is quite complex, as has been previously demonstrated under slightly different electrophoretic conditions (77). The monomer region appears to be a broad region encompassing 3 or 4 peaks; ν_2 appears with 2 or 3 peaks (77). Nonetheless, the increase of ν_1 as a function of digestion time is readily observed. The nature of the electrophoretic complexity of ν_1 is far from clear and cannot be entirely due to differential distribution of very lysine-rich histones (77), as discussed later in this paper.

The relative molar ratios of the major histone classes in isolated nuclei and in total ν_1, as determined by densitometry of stained SDS-electrophoretic gels (65), are presented in Table 1. Our analysis of SDS extracts from total chicken erythrocyte nuclei supports the concept of equimolar amounts of all the major histone classes (considering H5 and H1 as members of the same class), in contrast to a suggested model (63) and to our previous data on acid-extracted histones (64). However, isolated ν_1 reveals a considerable reduction of H5 and H1, a decrease of ~80–90% compared with intact nuclei. Qualitatively similar observations have been made by others (24, 34, 36). The slight decrease of H3 in total ν_1 (~20%) is very close to the limits of accuracy of densitometry. The analysis is further complicated by the fact that H3 and H2B migrate close to one another in our system.

Subfractionation of ν_1

In an effort to develop conditions favorable for the cyrstallization of ν_1, we have

Table 1. Molar ratios of the major histones

	Molar ratio*						
	Nuclei			ν Bodies			
Histone	Exp. 1	Exp. 2	$\bar{X}$	Exp. 1	Exp. 2	Exp. 3	$\bar{X}$
H4	1.00	1.00	1.00	1.00	1.00	1.00	1.00
H3	1.01	0.95	0.98	0.76	0.72	0.88	0.79
H2A	0.94	1.12	1.03	0.97	1.00	1.49	1.15
H2B	1.15	1.25	1.20	1.00	1.03	1.19	1.07
H5	0.92	0.93	0.93	0.13	0.15	0.11	0.13
H1	0.45	0.09	0.27	0.03	0.02	0.02	0.02

*Each column of histone molar ratios represents three separate gels on a single preparation. $\bar{X}$, represents the average of all data. Experience in our laboratory suggests that the molar ratios are accurate to ~±15%.

examined their solubilities in a large number of solvents containing inorganic and organic precipitants. One such experiment is shown in Fig. 2, which illustrates the relative effectiveness of KCl, $MgCl_2$, and $MnCl_2$ in precipitating ν_1. It is significant that divalent cations are capable of precipitating ~100% of ν_1, whereas KCl achieves only ~52% precipitation. These and other studies showed that different divalent cations (all as chloride salts) differ in their effectiveness (but all precipitate ~100% ν_1) as follows: Cu > Co, Zn, Mn > Mg.

The finding that ~50% of ν_1 is soluble in 0.1 M KCl buffer (KCl-soluble ν_1) allowed us to compare the chemical composition of these two subfractions. Preparative amounts of KCl-soluble ν_1 were obtained by dialysis of several milliliters of ν_1, overnight, versus 2 l of 0.1 M KCl buffer [0.10 M KCl, 0.2 mM EDTA, 0.1 mM PMSF, 0.2% isopropanol (pH 7.0)], at 4°C, followed by centrifugation for 10 min at 17,000 X g in a Sorvall RC2B. The resulting supernatant was either examined in the 0.1 M KCl buffer, or dialyzed stepwise against 2 l of 10 mM, 2 mM, and 0.2 mM EDTA, 12–18 hr per step. KCl-insoluble ν_1 was obtained from the centrifugation in 0.1 M KCl buffer, resolubilized by stepwise dialysis against EDTA solutions (as described above), and finally clarified by centrifugation for 10 min at 17,000 X g. Analysis of total ν_1 and the fractions of ν_1 on SDS-gel electrophoresis (Fig. 3A) revealed that the only major difference in histone

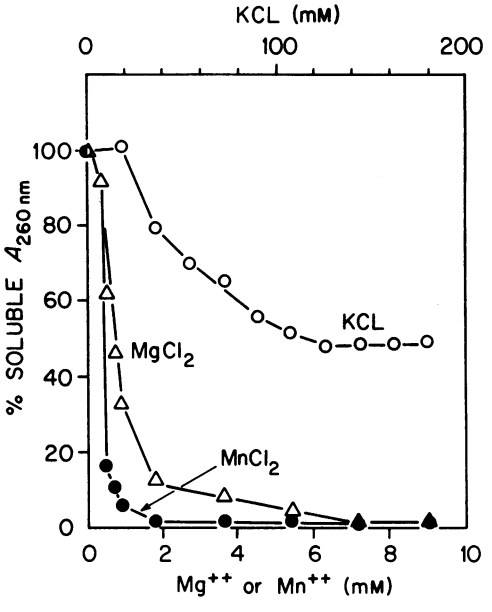

Figure 2. Solubility of total ν_1 as a function of KCl, $MgCl_2$, and $MnCl_2$ concentrations. Solutions contained: ν_1 = 1 mg/ml, 0.02 mM EDTA, and the indicated concentration of M^+Cl or $M^{2+}Cl_2$. After incubation for 16 hr at 4°, the solutions were centrifuged 10 min at 2000 X g, and A_{260} of supernatants was recorded.

composition was the complete absence of H5 and H1 in the KCl-soluble ν_1, whereas all of the residual H5 and H1 was present in the KCl-insoluble ν_1. The relative molar ratios of the other histones did not appear to differ significantly from the values reported for total ν_1 (Table 1).

Electrophoresis of the DNA fragments (Fig. 3B) revealed that the DNA obtained from KCl-soluble ν_1 is more homogeneous than that obtained from total ν_1 or KCl-insoluble ν_1. The DNA from KCl-soluble ν_1 revealed total absence of a peak [~125 nucleotide pairs (np)] migrating ahead of IA, while DNA from the KCl-insoluble ν_1 revealed a considerable enrichment of this DNA peak.

The very lysine-rich histones (H1 and H5) are known to greatly influence the solubility of chromatin (78–82). Nevertheless, it is surprising that even with the low level of H5 and H1 in the total monomers (reduced by ~80–90% compared with their content in total nuclei), ~50% of the monomers are precipitated in 0.1 M KCl. Because of the possibility of H5 and H1 migration in 0.1 M KCl, one cannot conclude from these experiments alone that the ~125-np DNA fragment is enriched with H5 and H1.

Electrophoretic analysis of the KCl-soluble and KCl-insoluble fractions of ν_1 on

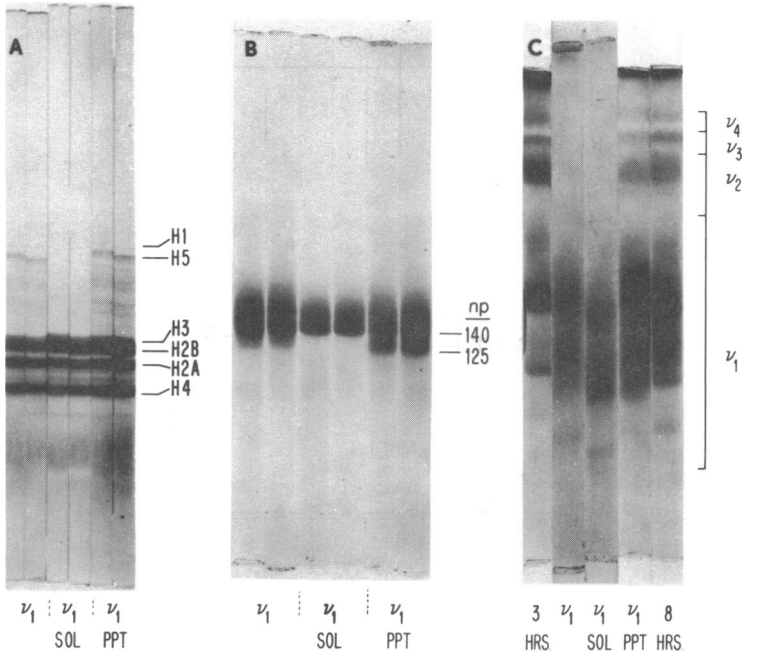

Figure 3. Electrophoretic comparisons of total ν_1, KCl-soluble ν_1, and KCl-insoluble ν_1. (A) Histone composition of total ν_1 and its subfractions as examined by SDS-gel electrophoresis. At the high sample loads employed in this study, several minor bands of unknown composition are readily visualized. The fastest broad band exhibited a robin's-egg blue color, similar to the D band of Fig. 4. (B) Electrophoresis of DNA fragments purified from total ν_1 and its subfractions. Assignment of the fragment sizes (140 and 125 np) is based upon parallel electrophoresis of calibrated standards (30). (C) Electrophoresis of the chromatin fragments — total ν_1, KCl-soluble ν_1, and KCl-insoluble ν_1 — compared with total digests of 3- and 8-hr duration.

acrylamide gels at low ionic strength reveals considerable complexity in both fractions (Fig. 3C). ν_1 which is soluble in 0.1 M KCl buffer exhibits two peaks of similar staining intensity and a weakly stained faster band; ν_1 which is insoluble in 0.1 M KCl reveals considerably greater complexity, including bands possibly attributable to ν_2, ν_3, ν_4, and ν_n.

In an effort to examine whether the electrophoretic complexity of ν_1 or KCl-soluble ν_1 can be correlated with histone composition, two-dimensional gel electrophoresis was employed. The first dimension was electrophoresis of the chromatin fragments at low ionic strength; the second, SDS-polyacrylamide electrophoresis (Fig. 4). Analysis of total ν_1 (Fig. 4A) revealed that the slower of the major monomer peaks contains all the histone fractions, whereas the faster major peak is devoid of H5, H1,

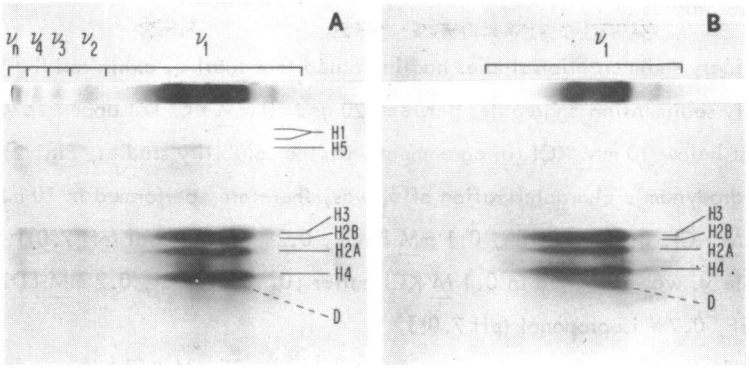

Figure 4. Two-dimensional gel electrophoretic comparison of: (A) total ν_1; and (B) KCl-soluble ν_1. Inserts at the origin of each slab gel show the first-dimension gels stained with amido black, with migration from left to right. Migration in the slab SDS electrophoretic gel is from top to bottom. At the high sample load of total ν_1, small amounts of ν_2, ν_3, ν_4, and ν_n can be observed. D, the diagonal blue band referred to in the text, probably represents fragments of DNA.

and some minor bands intermediate between H2B, H3, and H5. An unusual band (D) was observed diagonally crossing the H4 band. A differential distribution of very lysine-rich histones in the monomer peaks is entirely in accord with previous observations (77). Densitometric analysis of the major histone bands did not reveal any significant quantitative differences across the total ν_1 peak. KCl-soluble ν_1 did not reveal any H5, H1, or intermediate bands, yet still possessed electrophoretic heterogeneity of the monomer fragments. The D band was also very prominent. It stained a different shade of blue (i.e., robin's-egg blue) compared with the histones.

Although we do not know the chemical nature of the D band, we believe that it represents the monomer DNA fragments. If similar two-dimensional gels were stained with toluidine blue instead of amido black, only the D band stained; the histone bands were not seen. In addition, purified DNA fragments electrophoresed on 5% polyacrylamide gels exhibited considerable staining with amido black, whether or not the gels had been soaked in SDS sample buffer prior to staining. The fact that the D band is a continuous diagonal band implies that we are dealing with a homologous series of polymers consisting of either: (a) molecules of constant charge/unit length, but of varying length; or (b) molecules of constant size but of linearly varying charge. DNA of different chain lengths would be consistent with the first possibility. Clearly, definition of the nature of the D band awaits its isolation and characterization.

Hydrodynamic Studies of ν_1

Preliminary sedimentation studies had indicated that total ν_1 exhibited the formation of rapidly sedimenting aggregates between 20 and 50 mM KCl but appeared well-behaved at or below 10 mM KCl (in agreement with the solubility studies, Fig. 2). Extensive hydrodynamic characterization of ν_1 was, therefore, performed in 10 mM KCl buffer [10 mM KCl, 0.2 mM EDTA, 0.1 mM PMSF, 0.2% isopropanol (pH 7.0)]. Saline-soluble ν_1 was examined in 0.1 M KCl buffer [0.100 M KCl, 0.2 mM EDTA, 0.1 mM PMSF, 0.2% isopropanol (pH 7.0)].

Figure 5 presents representative experiments of ν_1 in 10 mM KCl buffer; data on saline-soluble ν_1 were very similar. Recorder traces during the sedimentation velocity runs indicated that the plateau region remained flat and the boundary symmetrical (Fig. 5A). Over the concentration range employed in the present study, the measured sedi-

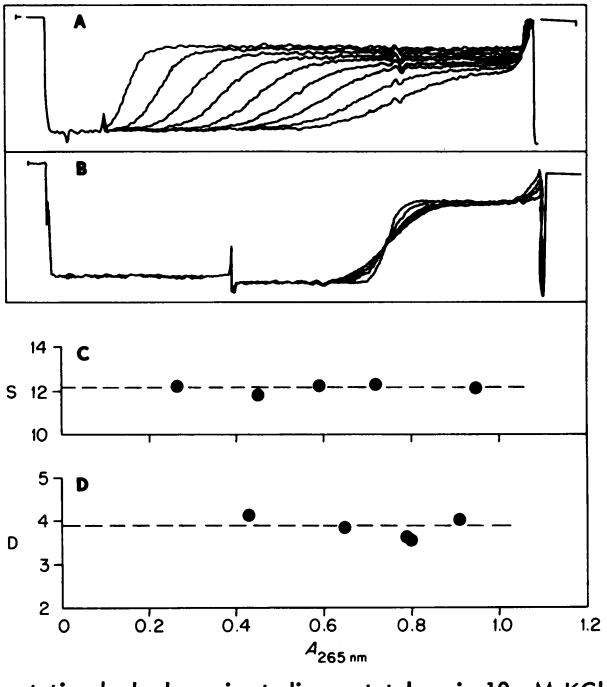

Figure 5. Representative hydrodynamic studies on total ν_1 in 10 mM KCl buffer. (A) Boundary-type sedimentation velocity run. Recorder scans taken every 32 min have been superimposed in this figure to demonstrate flatness in the plateau region and spreading of the sedimenting boundary. Sedimentation is from left to right, at 24,000 rpm in an An-G rotor. (B) Synthetic boundary-type diffusion measurement. Recorder scans taken every 16 min illustrate the boundary spreading and the absence of significant sedimentation. (C) Concentration-dependence of the sedimentation coefficient. (D) Concentration-dependence of the diffusion coefficient.

mentation and diffusion coefficients exhibited no concentration-dependence. The molecular parameters S° and D° presented for ν_1 and KCl-soluble ν_1 (Table 2) represent values averaged over the concentration range. Because S and D exhibited no concentration-dependence, only one concentration, within that region, was employed for study by equilibrium sedimentation; but the molecular weight was measured five times for each sample. The agreement between molecular weights calculated from the Svedberg equation and those measured by equilibrium sedimentation is quite good. The greater $\overline{M}_w$ for total ν_1 compared with KCl-soluble ν_1 could be due, in part, to the presence of some H5 and H1, and/or due to slight contamination with ν_2, ν_3, and ν_4.

Table 2 also presents computations of various molecular parameters of ν_1 and KCl-soluble ν_1, calculated by standard equations (83, 84). Two computations are

Table 2. Hydrodynamic data on ν_1

Parameter	Total ν_1 in 10 mM KCl buffer	KCl-soluble ν_1 in 100 mM KCl buffer
$S°_{20,w}$, Svedbergs	10.89 ± 0.28	11.41 ± 0.31
$D°_{20,w}$, Ficks	3.44 ± 0.13	3.90 ± 0.13
$\overline{v}$, ml/g	0.661 ± 0.006	(0.661 ± 0.006)*
$\overline{M}_w$ (S, D)	226,734 ± 11,095	209,547 ± 9,634
$\overline{M}_w$ (Equil)	230,432 ± 2,080	215,665 ± 2,086
$f_{20,w}$, g sec^{-1} $particle^{-1}$	1.176, 1.195†	1.037, 1.068†
d (anhydrous sphere), Å	78.0, 78.5	76.0, 76.7
f/fo	1.60, 1.61	1.44, 1.47
a/b, prolate axial ratio	11.1, 11.4	8.2, 8.7
a/b, oblate axial ratio	13.8, 14.3	9.7, 10.3
d (hydrated sphere), Å	124.5, 126.6	109.8, 113.1
δ_1 (maximum hydration), g H_2O/g ν_1	2.03, 2.11	1.33, 1.45

*$\overline{v}$ measured only for total ν_1; it is assumed to be the same for KCl-soluble ν_1.

†The first calculation of each pair is based upon $\overline{M}_w$ (S, D); the second, upon $\overline{M}_w$ (Equil).

presented for each parameter — the first calculated with $\overline{M}_w$ derived by the Svedberg equation; the second, from $\overline{M}_w$ derived by equilibrium sedimentation. It is of interest to note that the diameters of the equivalent hydrated sphere (~110 Å) and of the equivalent anhydrous sphere (~76 Å) are remarkably close to the first maxima of the small-angle X-ray scattering profiles for wet and dehydrated pellets of ν_1 (see below). It is very unlikely that the entire frictional coefficient ($f_{20,w}$) of ν_1 can be ascribed to particle asymmetry. Starting with that assumption, however, one can calculate the axial ratios of equivalent anhydrous prolate and oblate ellipsoids of revolution. Employing the parameters of KCl-soluble ν_1 throughout, the predicted prolate ellipsoid would be ~310 Å long X ~40 Å wide, and the oblate ellipsoid would be ~160 Å X ~17 Å thick. Both of these predicted structures are completely inconsistent with the electron microscopic data (see below). If the frictional coefficient is totally a consequence of hydration and increased volume, while remaining essentially spherical, one can calculate that hydration would amount to ~1.5 g H_2O/g anhydrous ν body.

Small-Angle X-Ray Scattering

Data on centrifuged pellets of total ν_1 in 10 mM KCl buffer and in 5 mM $MgCl_2$ are presented in Table 3. The positions of the X-ray reflections agree with those obtained by many authors (4, 47, 59, 87, 88) for pellets of isolated chromatin, nuclei, and mitotic chromosomes. Both pellets of ν_1 gave us the sharpest and clearest X-ray patterns of any samples we have so far examined.

Table 3. Small-angle X-ray scattering of ν_1

Buffer	Positions of maxima (Å)	
	Wet pellets	Vacuum-dried pellets
10 mM KCl buffer	107, 56.7, 36.6, (27)	84
5 mM $MgCl_2$	106, 57.8, 37	76, (35)

Electron Microscopy of ν_1

The reported diameters of dehydrated ν bodies in spread nuclei or chromatin have varied from 60 to 135 Å (4, 5, 8, 9, 11–13, 15). More recent data on isolated ν_1 bodies stained with dilute uranyl salts have yielded outer diameters (i.e., the diameter measured between the outer stained edges) of 81 ± 8 Å (5) and 85 Å (15). All of the measurements so far reported for ν bodies have been calibrated against grating replicas photographed at the same magnification. Unfortunately, the finest gratings available to us still have a minimum spacing of 4630 Å, or about 50 times greater than the diameter of ν_1. We have, therefore, chosen to carefully remeasure the outer and inner diameters of ν_1, when calibrated against the well-known lattice spacing of negative-stained catalase crystals (76). These data are presented for total ν_1, KCl-soluble ν_1, and total ν_1 after fixation for 10 min with 0.9% formaldehyde (pH 7.0) (Table 4). The outer diameter of 93–97 Å is somewhat larger than previously reported (5); the inner diameter of ~62 Å has not been explicitly measured before, but may correspond to early measurements (3) of a more classic negative stain. Table 4 establishes several important points: The morphology and dimensions of total ν_1 and KCl-soluble ν_1 are iden-

Table 4. Dimensions of ν_1 determined by electron microscopy

		Dimension of ν_1 (Å ± S.E.)		
Magnification	Region	Total ν_1	KCl-soluble ν_1	Total HCHO-fixed ν_1
240,000*	OD†	101.5 ± 3.1	98.6 ± 2.8	94.1 ± 2.7
296,000	OD	92.5 ± 2.6	89.0 ± 2.4	91.0 ± 2.9
Average‡	OD	97.0 ± 4.5	93.8 ± 4.8	92.6 ± 1.6
296,000	ID	61.7 ± 2.2	61.9 ± 2.0	

*Assuming $C_\alpha/2$ for catalase = 86 ± 2 Å (ref. 76), the final plate magnifications were 240,000 ± 6,200 and 296,000 ± 7,500.

†OD, outer diameter, measured between the outer stained edges; ID, inner diameter, measured between the inner stained edges.

‡Average of diameters measured at the two different magnifications not weighted to the numbers of particles measured at each magnification.

tical, within error. Formaldehyde fixation of ν_1 has no significant effect on particle diameter, in contrast to a previous report (9). We have also measured particles dried from stain without Photo-flo and find no appreciable difference in their morphology and dimensions.

DISCUSSION

The availability and ease of storage of large amounts of chromatin ν_1 by zonal ultracentrifugation permits extensive biophysical characterization of a well-defined and reproducible preparation. We have demonstrated that dialysis of total chicken erythrocyte ν_1 against buffers containing 0.1 M KCl permits subfractionation of the preparation into KCl-soluble ν_1 and KCl-insoluble ν_1. The KCl-soluble ν_1 can be examined by hydrodynamic techniques at solvent conditions (i.e., 0.1 M KCl) that would be expected to exhibit less significant charge effects than the lower-ionic-strength solvents [i.e., 10 mM KCl or 10 mM Tris (25, 60)] employed for the study of total ν_1. These KCl-soluble monomers closely resemble the "core" particles that have been examined in detail by low-angle neutron scattering (60) as well as by preliminary hydrodynamic characterization (25).

The analytical data presented here support the thesis that the KCl-soluble ν_1 consist of equimolar amounts of H4, H3, H2A, and H2B associated with DNA of 130–140 np. From the measured molecular weights of KCl-soluble ν_1 (i.e., 210,000–216,000 daltons) and its constituent DNA (i.e., ~94,000 daltons, estimated by gel electrophoresis comparison with calibrated DNA fragments), one obtains a calculated protein molecular weight per ν_1 of 116,000–122,000 daltons and an estimated protein/DNA (wt/wt) of 1.24–1.30. Since the sum of the molecular weights of H4 + H3 + H2A + H2B equals 55,400, the analytical data support the view of pairs of histones per ν_1 (3, 63). The KCl-soluble ν_1 are completely devoid of H5 and H1, whereas the measured histone molar ratios of total chicken erythrocyte nuclei are consistent with equimolar amounts of all the histone classes, including the very lysine-rich histones (H5 and H1).

The frictional coefficient measured by hydrodynamic techniques is a function of both particle asymmetry and extent of hydration. The present electron microscopic studies and published neutron-scattering data (58, 60) support the view that the particle asymmetry is small. If the KCl-soluble (or "core") ν_1 are indeed spherical (i.e., $f/f_o = 1$) the estimated degree of hydration would be 1.33–1.45 g H_2O/g anhydrous ν

body. Although this value is considerably higher than hydration values of proteins and nucleic acids (83, 84), it is remarkably similar to estimates of the hydration of E. coli 30 and 50 S ribosomal subunits (87) (30 S particles, 1.27–1.39 g H_2O/g dry particles; 50 S, 1.35–1.38 g H_2O/g dry particles). It appears that both ν bodies and ribosomes are considerably hydrated structures.

The KCl-soluble ν_1 are calculated to have a diameter of an equivalent hydrated sphere of 110–113 Å, very close to the overall diameter of a model spherical particle (106 Å), estimated from low-angle neutron scattering (ref. 60, their model A). We have previously (61, 65, 88) reported that centrifuged pellets of ν_1 exhibit the series of small-angle X-ray scattering maxima characteristics of native nuclei and chromatin, and have interpreted these data in terms of various arrays of close-packed spherical particles (61). The similar values for the diameter of the hydrated sphere, and the first-order X-ray maximum support the contention that the ~110 Å X-ray peak arises from the lattice of the close-packed ν_1; i.e., ν_1 are packed to a center-center distance of ~110 Å. Similarly, the estimated value of the diameter of an equivalent anhydrous sphere (76–77 Å) is extremely close to the first-order small-angle X-ray peak observed for dried chromatin or dried pellets of ν_1 (76–84 Å). Such a correspondence of data would support the view that during dehydration of chromatin, or pellets of ν_1, the particles shrank to an average diameter of ~80 Å, while remaining largely close-packed (61).

We (5) and others (15) have pointed out the possibility that the uranyl staining by the ν bodies and the "connecting strand" could represent binding to (and localization of) the chromatin DNA. We believe that the data presented in this study on particle dimensions observed by electron microscopy constitute our most accurate determinations. From our measurements the "shell" of peripheral uranyl staining was ~16–18 Å thick, or about as wide as double-stranded DNA, while the unstained "core" was ~62 Å in diameter. Since the uranyl-stained ν_1 have an outer diameter significantly greater than the ~80 Å estimated for anhydrous particles, it remains conceivable that the shape of the particle as examined by electron microscopy is slightly different than when the particle is dehydrated in a mass of chromatin. Alternatively, ν_1 may be slightly asymmetric (e.g., an oblate ellipsoid) yet close-pack in the dry state to an average center-to-center distance of ~80 Å. The model previously proposed for the "protein core" of ν_1 (65) — close-packing of the globular regions of the constituent histones, with point-group symmetry, and generating a true dyad axis — is, in fact, slightly oblate.

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