Fractionation of Purified Nucleosomes on the Basis of Aggregation Properties†

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ABSTRACT: Monomeric nucleosomes from micrococcal nuclease digests of rat liver nuclei have been purified on Sepharose columns in the presence of divalent cations and 0.6 M NaCl. The particles contain histones H2A, H2B, H3, H4, and a complement of nonhistone chromosomal proteins. In 0.6 M NaCl, the nucleosome mixture sedimented at 10 S; however, when the NaCl was removed approximately 30% of the particles aggregated and precipitated, and the remaining soluble fraction sedimented at 11 S. The aggregation phenomenon was divalent cation-dependent and reversible. Characterization of the macromolecular components of the subfractions of nucleosomes showed that the subfractions differed in composition of species of histone H3 as well as of several nonhistone chromosomal proteins but not in the size of the DNA fragment present. The aggregation properties of the isolated nucleosomes showed similarities to the divalent cation-dependent differences in the extent of chromatin condensation in the intact eukaryote nucleus.

Morphologically, the interphase nucleus in eukaryote organisms is organized into more condensed and less condensed regions that roughly correlate with transcriptionally inactive and active areas of the nucleus (Littau et al., 1964). Although the molecular basis is not understood, the differentiation is preserved in nuclei isolated in the presence of divalent cations (Schneider and Petermann, 1950). Dilution or chelation causes it to be lost and the nuclei to become morphologically homogeneous (Ris and Mirsky, 1949; Anderson and Wilbur, 1952; Mazia, 1954: Philpot and Stanier, 1956; Davies and Spencer, 1962; Olins and Olins, 1972; Lezzi and Robert, 1972; Chevaillier and Philippe, 1973).

Recent evidence that more than 95% of the nuclear DNA is complexed with histones in particulate structures called nucleosomes (Noll, 1974; Kornberg, 1974; Olins and Olins, 1974; Van Holde et al., 1974) suggests the more and less condensed morphological features of the nucleus may reflect differences in the macromolecular composition and/or the packing of the nucleosomes. For the purpose of probing these questions, we have developed a procedure for isolating monomeric nucleosomes from micrococcal nuclease digests of rat liver nuclei under ionic conditions chosen to preserve any properties of individual particles relevant to differences in the extent of chromatin condensation in intact nuclei. This procedure differs from conventional procedures for isolation of nucleosomes (Bakayev et al., 1975; Honda et al., 1975; Rill and Van Holde, 1973; Varshavsky et al., 1976) in that divalent cations were present throughout. Characterization of the particles isolated by this procedure showed that at least two major classes of nucleosomes were present which differed in their properties of self-aggregation.

Experimental Procedure

Materials. Sepharose 4B was purchased from Pharmacia, acrylamide from Bio-Rad, and micrococcal nuclease from Worthington.

Isolation of Nuclei. Nuclei were isolated by the method of Chevaillier and Philippe (1973) from fresh rat livers. Following scissor-mincing, each liver was homogenized with a motor-driven Potter-Elvehjem homogenizer in 150 mL of 1 mM Tris,[1] 25 mM KCl, 0.9 mM $MgCl_2$, 0.9 mM $CaCl_2$, 0.14 mM spermidine, pH 7.6 (C buffer) containing 2.2 M sucrose. The homogenate was filtered through four layers of cheesecloth and centrifuged in a Spinco 42 rotor at 25 000 rpm and 4 °C for 1 h. The supernatant fraction was poured off and the inside of the tube wiped with a tissue. The nuclear pellets from one liver were resuspended with a homogenizer in 5.0 mL of C buffer.

Preparation of Nucleosomes. The nuclei from one rat liver (DNA concentration 1–2 mg/mL) were treated with 200 units/mL of micrococcal nuclease in a total volume of 5.0 mL of C buffer containing 2.4 mM Mg^{2+} (instead of 0.9 mM) at 37 °C for 30 min. The digestion was stopped by the addition of 0.5 mL of 10 mM EGTA and dialyzed at 4 °C against C buffer containing 0.25 M sucrose and 0.6 M NaCl. After 20 h of dialysis, 2.5 mL of the dialysate was applied to a 2.5 × 90 cm column of Sepharose 4B equilibrated with the same buffer at 4 °C to separate the nucleosomes from unbound nuclear proteins. The second half of the dialysate was applied to the same Sepharose column 24–48 h later. The nucleosomes purified by this procedure were stable for several weeks stored at 4 °C in C buffer plus 0.6 M NaCl and 0.25 M sucrose. Properties of the nucleosomes did not vary either with length of time of dialysis or from preparation to preparation (14 preparations have been characterized).

Preparation of nuclei and of nucleosomes in the presence of 1 mM $PhCH_2SO_2F$ (Heinrich et al., 1976) to prevent proteolysis made no detectable differences in either the properties or the macromolecular composition of nucleosomes isolated by this method.

Sucrose Gradient Experiments. The sedimentation behavior of purified nucleosomes was analyzed on 11-mL linear 10–30%

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[1] Abbreviations used are: EGTA, ethylene glycol bis(β-aminoethyl ether)-*N,N'*-tetraacetic acid; EDTA, (ethylenedinitrilo)tetraacetic acid; Tris, 2-amino-2-hydroxymethyl-1,3-propanediol; ν, nucleosomes; S, soluble fraction of nucleosomes; Ag, aggregated fraction of nucleosomes; UV, ultraviolet.

sucrose gradients formed in C buffer plus the indicated amount of NaCl. 0.2–0.5 mL of the sample was layered onto the gradients and they were centrifuged in a Spinco SW41 rotor at 35 000 rpm for 18 h at 4 °C. The gradients were pumped out from the bottom via a capillary tube carefully inserted from the top. 0.8-mL fractions were collected and the absorbance at 260 nm was measured.

Analytical Ultracentrifugation. The sedimentation coefficients of purified nucleosomes in C buffer containing 0.6 M or no NaCl and no sucrose were measured in a Spinco Model E ultracentrifuge equipped with UV optics. $s_{20,w}$ was calculated using solvent viscosities determined in a capillary viscometer and a partial specific volume ($\bar{v}$) of 0.69 cm^3/g (Sahasrabuddhe and Van Holde, 1974).

Stokes Radius Determination. The Stokes radius (R_0) of the purified nucleosomes in C buffer containing 0.6 M NaCl or no salt was determined by the method of Siegel and Monty (1966) using columns of Sepharose 4B and *Escherichia coli* β-galactosidase (R_0 = 69 Å (Sund and Weber, 1963)), beef liver catalase (R_0 = 52 Å (Sumner and Gralen, 1938)), and yeast alcohol dehydrogenase (R_0 = 46 Å (Hayes and Velick, 1954)) as standards. V_0 and V_i were determined using phage T4 and ^3H_2O, respectively. Catalase was monitored by its absorbance at 410 nm. β-Galactosidase and alcohol dehydrogenase activities were measured by the methods of Ullmann et al. (1968) and Vallee and Hoch (1955), respectively. The elution position of the nucleosomes was measured by absorbance at 260 nm.

Molecular-Weight Calculations. The molecular weight (M) of the purified nucleosomes was calculated using the equation

$$M = 6\pi\eta N R_0 s/(1 - \bar{v}\rho)$$

where η = solvent viscosity, N = Avogadro's number, R_0 = Stokes radius, s = sedimentation coefficient, $\bar{v}$ = partial specific volume, and ρ = density of the solvent.

Gel Electrophoresis. For size analysis of the DNA present in the fractionated nucleosomes, the DNA was purified by the method of Noll (1974) and 10 μg of each of the samples was electrophoresed on 2.5 or 6% acrylamide slab gels (Loening, 1967) at 25 mA for 2.5 h and compared with DNA extracted from a partial micrococcal nuclease digest of rat liver nuclei containing monomer, dimer, and higher oligomer nucleosome forms. The gels were stained with 2 μg/mL ethidium bromide in water for 30 min and photographed on a UV light box (UV Products, San Gabriel, Calif.) with a Kodak 23A filter.

Total proteins of pooled and fractionated nucleosomes were analyzed on 12.5% discontinuous sodium dodecyl sulfate–acrylamide slab gels (Laemmli, 1970). Samples were prepared by precipitation at −20 °C for 4 h with 2 volumes of 95% ethanol. After centrifugation, the supernate was removed and the precipitate was dissolved in the loading buffer, heated in a boiling water bath for 60 s, and applied to the gel. Electrophoresis was at 60 V for 45 min, and then 120 V for 2 h and 45 min.

Triton–urea–acetic acid–acrylamide slab gels for the analysis of histones and other acid-soluble proteins were prepared as described by Cohen et al. (1975) and Alfageme et al. (1974) using 0.38% Triton X100. Histones were extracted for electrophoresis by a modification of the method of Dingman and Sporn (1964). Samples of nucleosomes containing 0.6 A_{260} units were brought to 2 M in NaCl and allowed to stand at 4 °C for 30 min. One-tenth volume of 1 N HCl was added and the precipitated DNA and nonhistone proteins were removed by centrifugation. The supernatant fraction was dialyzed against 0.01 N HCl, lyophilized, and dissolved in 10 μL of sample buffer for application to the gel. Electrophoresis was at 200 V for 8 h. The gels were stained as described by Laemmli (1970) using Coomassie blue.

Results

The purpose of these experiments was to study the properties of nucleosomes isolated under conditions chosen to preserve structural features related to differential chromatin condensation in the intact nucleus. Chevaillier and Philippe (1973) had developed conditions for isolating nuclei from mouse liver in the presence of Ca^{2+} and Mg^{2+} which preserved the native chromatin morphology under the electron microscope. The enzyme used for digestion of the nuclei, micrococcal nuclease, is active only in the presence of Ca^{2+} and at ionic strengths below 0.2 (Cuatrecasas et al., 1967; Sanders, unpublished observations). Thus, nucleosomes could be isolated with Mg^{2+} present at all times if the micrococcal nuclease digestion was stopped by selective chelation of Ca^{2+} using EGTA.

Isolation of Nucleosomes. Purified nuclei were digested to the stage where more than 90% of the DNA migrated on acrylamide gel electrophoresis as monomer fragments. Approximately 20% of the DNA was acid soluble at this point. The digestion was stopped with EGTA and the nucleoprotein complex was solubilized by dialysis overnight against C buffer containing 0.25 M sucrose and 0.6 M NaCl. Nucleosomes were separated from H1 and other nuclear proteins by gel filtration on a column of Sepharose 4B. The first major peak eluted from this column contained nucleic acid and protein (Figure 1A). The major protein components of this material were the histones H2A, H2B, H3, and H4 (Figure 1B). The majority of the nonhistone nuclear proteins and H1 were eluted in the second major peak which contained only a small amount of 260-nm absorbing material.

Fractionation of Purified Nucleosomes. Dialysis of the purified nucleosomes pooled as indicated in Figure 1A against C buffer containing 0.25 M sucrose and no NaCl reproducibly resulted in the aggregation and precipitation of approximately 30% of the nucleosomes. The dependence of this aggregation phenomenon on NaCl concentration was initially investigated by studying the sedimentation behavior of the nucleosomes following dialysis against different concentrations of NaCl. Purified monomeric nucleosomes were quantitatively recovered in a single peak sedimenting at about 10 S from gradients formed in C buffer containing 0.6 M NaCl (Figure 2A). As the NaCl concentration was reduced to 0.2 M, the sedimentation rate was increased to 11 S and 5–10% of the material aggregated and was recovered in the pellet. When all the NaCl was removed, 30% of the nucleosomes aggregated and precipitated. The remaining soluble material sedimented as a broadened peak with the major portion at 11 S.

The aggregation behavior, but not the shift in sedimentation rate, was shown to be dependent on the presence of divalent cations. When the experiment was repeated with 10 mM EDTA present in the buffers, all of the A_{260} applied to the gradient remained soluble and was recovered in the 11S peak, as shown in Figure 2B.

Both the aggregation phenomena and the shift in sedimentation rate were reversible. This was shown by fractionating purified nucleosomes into soluble (S) and aggregated (Ag) forms by centrifugation after dialyzing away the NaCl as before. The NaCl concentration of both samples was readjusted to 0.6 M (this dissolved the Ag fraction) and sedimentation of the S and Ag samples separately through sucrose gradients containing 0.6 M NaCl showed that both sedimented at the

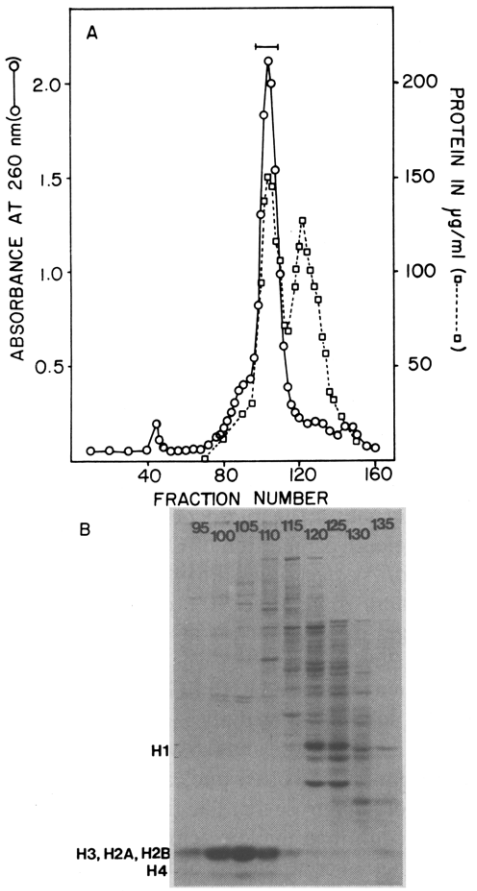

FIGURE 1: Purification of monomer nucleosomes by gel filtration on Sepharose 4B. 2.5 mL of a digest of rat liver nuclei that had been dialyzed against C buffer plus 0.6 M NaCl and 0.25 M sucrose was applied to a 2.5 × 90 cm column of Sepharose 4B equilibrated in the same buffer at 4 °C. 2.4-mL fractions were collected at a flow rate of 11.3 mL/h. (A) (O–O) Elution profile measuring absorbance at 260 nm; and (□- - -□) protein determined by the method of Lowry et al. (1951). The fractions included under the bar were pooled for further characterization. (B) Discontinuous sodium dodecyl sulfate–acrylamide gel electrophoresis of 0.2-mL aliquots of indicated fractions from the column.

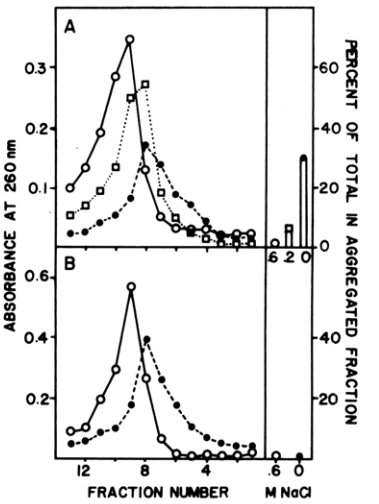

FIGURE 2: Changes in the sedimentation and aggregation behavior of nucleosomes with changes in ionic conditions. Purified nucleosomes in C buffer containing 0.25 M sucrose and 0.6 M NaCl were dialyzed against the indicated buffer, all with 0.25 M sucrose present. 0.2 mL of the dialysate was loaded onto 11 mL 10–30% linear sucrose gradients prepared in the buffer against which the sample had been dialyzed and the gradients were centrifuged in a Spinco SW 41 rotor at 4 °C for 18 h at 35 000 rpm. Sedimentation was from left to right. The absorbance at 260 nm of each 0.8-mL sample collected from the bottom was determined. The bar graph at the right indicates the percentage of each fraction recovered in the pellet. (A) (O–O) Control dialyzed against C buffer plus 0.6 M NaCl; (□ · · · □) dialysis against C buffer plus 0.2 M NaCl; (●- -●) dialysis against C buffer containing no NaCl. (B) (O–O) Pooled nucleosomes dialyzed against C buffer containing 10 mM EDTA and 0.6 M NaCl; (●- - -●) dialysis against C buffer containing 10 mM EDTA and no NaCl.

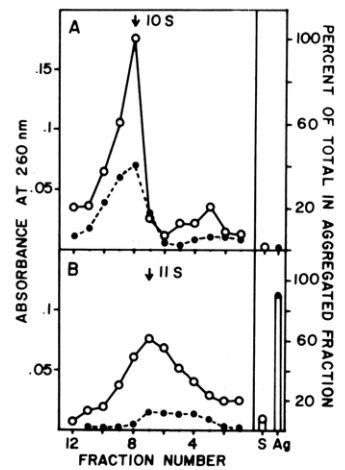

FIGURE 3: Reversible nature of the aggregation properties of the soluble (S) and aggregated (Ag) fractions of nucleosomes. S and Ag subfractions were prepared in quantity by dialysis of pooled nucleosomes against C buffer plus 0.25 M sucrose followed by centrifugation in a Type 50 rotor at 30 000 rpm for 1 h. The supernatant and aggregated fractions were separately dialyzed against C buffer containing 0.6 M NaCl and 0.25 M sucrose and each was divided. (A) Half was sedimented through 10–30% sucrose gradients prepared in C buffer plus 0.6 M NaCl as in Figure 2. (O–O) S fraction in C buffer plus 0.6 M NaCl; (●- - -●) Ag fraction in C buffer plus 0.6 M NaCl. (B) The NaCl was removed from the other half by dialysis against C buffer containing 0.25 M sucrose. These samples were sedimented through 10–30% sucrose gradients in C buffer. (O–O) S fraction readjusted by dialysis back to 0 NaCl; (●- - -●) Ag fraction reaggregated by dialysis against C buffer containing no salt.

same rate as the original 10S mixture (Figure 3A). Subsequent readjustment of the salt concentration of these S and Ag fractions from 0.6 M to no NaCl by dialysis resulted in the previously observed shift in sedimentation rate of the S fraction from 10 to 11 S and complete recovery of the sample in the soluble form (Figure 3B). The Ag fraction was 90% precipitated by removing the 0.6 M NaCl. The reversibility of the aggregation phenomenon when the S and Ag particle types were separated indicated that the ability to aggregate is an inherent property of the Ag particles themselves.

Sedimentation Rate and Stokes Radius Measurements. The question of whether the shift in sedimentation rate of the nucleosomes with changes in NaCl concentration represented a molecular weight change or a change in molecular shape or both was investigated by measuring the sedimentation coefficient in the analytical ultracentrifuge and the Stokes radius by gel filtration on Sepharose columns (Siegel and Monty, 1966). $s_{20,w}$ values in the presence of 0.6 M NaCl were lower than in its absence (Table I). Gel filtration of the nucleosome pooled material in 0.6 M NaCl and the S fraction in no salt compared with globular proteins of known Stokes radii gave the results shown in Figure 4. The nucleosomes became more compact in low salt with a decrease in Stokes radius from 74 Å in 0.6 M NaCl to 68 Å in no salt (Table I). The molecular weights calculated from these data for both the pooled nucleosomes and the S fraction are consistent with the interpretation that the shift from 10 to 11 S on lowering the salt concentration is due to a change in shape and not a change in

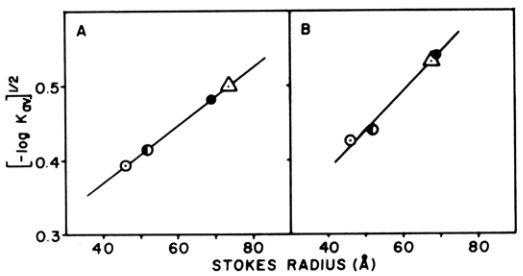

FIGURE 4: Determination of Stokes radius of nucleosomes in 0.6 M NaCl and no NaCl. Sepharose 4B columns equilibrated in C buffer and either 0.6 M or no NaCl were calibrated using yeast alcohol dehydrogenase, beef liver catalase, *E. coli* β-galactosidase, phage T4, and ^3H_2O as markers. Then a mixture of phage T4, nucleosomes, and ^3H_2O was applied to each column with elution of the nucleosomes monitored at 260 nm. $(-\log K_{av})^{1/2}$ was calculated for each protein as described by Siegel and Monty (1966). (A) $(-\log K_{av})^{1/2}$ plotted against Stokes radius for pooled nucleosomes and the marker proteins in C buffer plus 0.6 M NaCl. (⊙) Yeast alcohol dehydrogenase; (◐) beef liver catalase; (●) *E. coli* β-galactosidase; (△) nucleosomes. (B) $(-\log K_{av})^{1/2}$ plotted against Stokes radius for the S fraction and the marker proteins in C buffer containing no NaCl.

TABLE I: Composition and Hydrodynamic Properties of Pooled Nucleosomes and the S and Ag Subfractions in the Presence and Absence of 0.6 M NaCl.

		NaCl Present in C Buffer	
Property	Preparation[a]	0.6 M	None
$s_{20,w}$	Nucleosomes (a)	9.7	
	Nucleosomes (b)	9.9	
	S (a)		11.6
	S (b)		10.9
	Ag (a)	9.7	
Stokes radius	Nucleosomes (b)	74 Å	
	S (b)		68 Å
Mol wt	Nucleosomes (b)	268 000	
	S (b)		271 500
Protein:DNA (wt ratio)	Nucleosomes (b)	2.1:1	

[a] a and b indicate data collected on two different preparations.

molecular weight (see Table I). The molecular weight for the monomeric nucleosome is in good agreement with the estimate of 260 000 from electron microscopic data (Kornberg, 1974) and the hydrodynamic measurement of 289 000–330 000 for formaldehyde-fixed particles from chicken erythrocyte nuclei (Senior et al., 1975).

Analysis of the Composition of Nucleosomes, S and Ag Fractions. The differences in aggregation behavior of the S and Ag fractions implied differences in either the protein or nucleic acid content of the particles. Therefore, the histone, nonhistone chromosomal protein, and DNA contents were compared by electrophoretic techniques. Analysis of the DNA purified from the S and Ag fractions (Figure 5A) by electrophoresis on 2.5% acrylamide slab gels showed no differences in the molecular weight of the DNA present. To be certain that the fragment size of the DNA was not an important factor in determining aggregation behavior, we digested purified nucleosomes with micrococcal nuclease. DNA extracted from the redigested particles fell into the size classes described by other workers for limit digests (Axel et al., 1974; Weintraub, 1975). The redigested particles were separated from the enzyme and nucleotides by gel filtration on a Sepharose 4B column in C buffer plus 0.6 M NaCl. Velocity sedimentation of the redigested

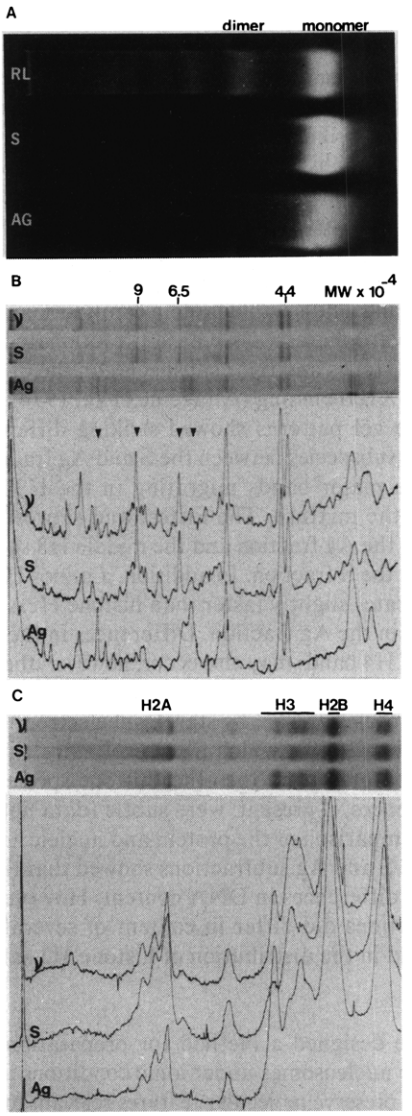

FIGURE 5: Electrophoretic analysis of the DNA and protein components of the pooled nucleosomes and the S and Ag subfractions. Electrophoresis was from left to right in all cases. (A) Electrophoresis of DNA extracted from the nucleosome subfractions compared with DNA extracted from a partial micrococcal nuclease digest of rat liver nuclei on 2.5% acrylamide gels (Loening, 1967) stained with ethidium bromide and photographed under UV light. From top to bottom: RL, DNA extracted from a partial digest of rat liver nuclei showing monomer, dimer, and oligomeric nucleosomes; S, DNA extracted from the S fraction; Ag, DNA extracted from the Ag fraction. (B) Total proteins present in pooled nucleosomes and the S and Ag subfractions analyzed by 12.5% discontinuous sodium dodecyl sulfate–acrylamide gel electrophoresis (Laemmli, 1970). The portion of the gel where the histones appear is not shown. Densitometer tracings of the negative are shown below the photograph of the gel. From top to bottom: ν, pooled nucleosomes; S, the soluble subfraction; Ag, the aggregated subfraction. The arrows indicate proteins which are clearly distributed in either the S or the Ag subfraction. (C) Acid-soluble proteins present in pooled nucleosomes and the S and Ag fractions. Histones were extracted from the samples and applied in 10 μL to triton–urea–acetic acid–acrylamide slab gels prepared as described by Cohen et al. (1975) and Alfageme et al. (1974). The various histone species are indicated by bars. From top to bottom: ν, pooled nucleosome histone; S, histones extracted from the soluble subfraction; Ag, histones extracted from the aggregated subfraction. Densitometer tracings of the negative are shown below the photograph.

particles in 0.6 M NaCl or in no salt showed no change in either the shift in sedimentation rate of the S fraction or in the amount of the Ag fraction recovered (data not shown).

The protein content of the nucleosome mixture and the S and Ag subfractions was approximately 30% (w/w) acid-in-

soluble protein and 70% histone. Individual protein species associated with the mixture and the two subfractions were compared by discontinuous sodium dodecyl sulfate–acrylamide slab gel electrophoresis. In addition to the nucleosome histones which are not well resolved under these conditions, a number of prominent nonhistone chromosomal proteins were present in the mixture (Figure 5B). Of these, some were associated in similar amounts with both the soluble and aggregated subfractions, but several protein species were present in only one subfraction. These are indicated with arrows in Figure 5B.

Histone and acid-soluble protein composition was analyzed with an electrophoresis system that fractionated all four nucleosome histones and their subspecies on the basis of charge and Triton X100 binding (Alfageme et al., 1974; Cohen et al., 1975). The gel patterns showed striking differences in the histone H3 subspecies between the S and Ag fractions (Figure 5C). Three major bands migrating in the H3 region were present in the mixture. The fastest and slowest bands were enriched in the Ag fraction and the middle H3 subspecies was enriched in the S fraction. In addition, a minor H3 subspecies which migrates slightly faster than histone H2A appeared to be missing in the Ag fraction. Differences in the relative distribution of H4 bands may also occur; however, the doublet was not always well resolved in this electrophoresis system. Analysis of the H4 species present by starch gel electrophoresis (Sung and Smithies, 1969), which also resolves rat liver H4 as a doublet separated from the other histone species, suggested that differences, if present, were subtle (data not shown).

Thus, comparison of the protein and nucleic acid compositions of the S and Ag subfractions showed that there were no significant differences in DNA content. However, the S and Ag subfractions did differ in content of several nonhistone proteins and in the distribution of histone H3 subspecies.

Discussion

We have designed a method for preparation of purified monomeric nucleosomes under ionic conditions that could be expected to preserve molecular features relevant to the divalent cation-dependent differences in condensation of interphase chromatin. The procedure differs from other methods of nucleosome preparation in several ways: (1) the digestion with micrococcal nuclease took place when the nuclei were intact; (2) the nucleoprotein complex was solubilized completely using 0.6 M NaCl so that the H1 was completely removed and the material characterized represented all of the nucleosome structures present in the nucleus; (3) the nucleosomes were isolated in the presence of divalent cations so that the structural features related to differences in chromatin condensation might be retained. In other studies, nucleosomes have been isolated from micrococcal nuclease digests of chromatin prepared in the absence of divalent cations (Rill and Van Holde, 1973; Varshavsky et al., 1976; Honda et al., 1975; Rill et al., 1975; Bakayev et al., 1975). These procedures have yielded particles heterogeneous with respect to H1 content (Varshavsky et al., 1976; Honda et al., 1975) that may simply be a result of the known tendency of H1 to dissociate when chromatin is digested with nucleases (Chae, 1974).

The monomeric nucleosomes we have characterized have an interesting property which may correlate with differences in the extent of interphase chromatin condensation. When the 0.6 M NaCl was removed from purified nucleosomes, approximately 30% of the particles aggregated and precipitated. The aggregation phenomenon was divalent cation-dependent; when EDTA was added to the buffer system in excess over the Ca^{2+} and Mg^{2+} ions, aggregation did not occur. Similarly, the removal of divalent cations from isolated nuclei causes the morphologically condensed parts of the chromosome structure to decondense and become homogeneous in appearance (Ris and Mirsky, 1949; Anderson and Wilbur, 1952; Mazia 1954; Philpot and Stanier, 1956; Davies and Spencer, 1962; Olins and Olins, 1972; Lezzi and Robert, 1972; Chevaillier and Philippe, 1973). At this time, the correlation between the divalent cation-dependent aggregation of nucleosomes reported here and the differences in the extent of chromatin condensation in the intact nucleus is only circumstantial. However, the parallels are striking. Electron-microscopic studies of the morphology of the chromosome fiber have shown that the fiber diameter doubles in the presence of divalent cations (Ris, 1967; Pooley et al., 1974). Finch and Klug (1976) have observed the divalent cation dependence of formation of a "solenoid" structure in isolated chromatin in the electron microscope that they propose to be the mechanism for the increase in fiber width. Their model requires divalent cation-dependent bivalent interaction of nucleosomes with each other in order to stabilize the coiled structure. The aggregation phenomenon reported here also requires that the Ag particles have two sites for nucleosome interaction. This condition may be met if each nucleosome consists of symmetrical tetramers of the histones as proposed by Weintraub et al. (1975).

When the NaCl was removed from the mixture of nucleosomes, the nonaggregated fraction underwent a conformational change with an increase in sedimentation velocity from 9.6 to 10.9 S and a decrease in Stokes radius from 74 to 68 Å. The molecular weight determined for both conformational states was 270 000. The transition occurred between 0.6 and 0.2 M NaCl and may correspond to the structural transition observed by nuclear magnetic resonance in chromatin in this range of NaCl concentrations (Bradbury et al., 1973, 1975).

A comparison of the macromolecular composition of the soluble (S) and aggregated (Ag) fractions showed no detectable differences in the size of the DNA fragments present. Redigestion of the isolated nucleosomes to a point where the DNA was very heterogeneous in size gave no change in the aggregation properties. This observation suggested that the DNA available for nuclease attack does not play a role in the aggregation of the particles. On the other hand, comparison of the proteins present in the soluble and aggregated fractions showed significant differences in both the nucleosome histones and nonhistone chromosomal proteins. Some nonhistone proteins were common to both the S and Ag subfractions; however, several major proteins were present in either the S or the Ag particles but not in both. The major subspecies of H3 present in the mixture were asymmetrically distributed between the S and Ag particles with two subspecies enriched in the Ag fraction and one predominating in the S fraction. The differences in protein composition between the S and Ag particles suggest that the protein components of the nucleosomes are responsible for the differences in aggregation behavior.

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