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Biochemistry

Subunit structure of chromatin and the organization of eukaryotic highly repetitive DNA: Nucleosomal proteins associated with a highly repetitive mammalian DNA

(*Eco*RI restriction endonuclease/nucleosome fractionation/mammalian repetitive DNA sequences/histone and nonhistone proteins)

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ABSTRACT **Component α DNA is a homogeneous, highly repetitive fraction that comprises nearly a quarter of the African green monkey (*Cercopithecus aethiops*) genome. By restriction enzyme analysis, it has a repeat periodicity of 176 ± 4 nucleotide base pairs, corresponding closely with the length of DNA contained within a nucleosome. The sequence is organized into large blocks of constitutive heterochromatin. A method is described here for the isolation of intact polynucleosomal arrays containing only component α sequences. Isolated monkey nuclei are treated with *Eco*RI, which releases only component α nucleosomal arrays; the arrays are then fractionated and purified by sedimentation in sucrose gradients. The method permits a compositional analysis of the proteins associated with a constitutively repressed, heterochromatic sequence.**

The major differences in the proteins associated with component α nucleosomes that distinguish them from the bulk DNA nucleosomes are a decrease in the content of the H1 histones in the component α nucleosomes and a concomitant increase in the amount of certain nonhistone proteins. The specific observations are: (*i*) In the component α nucleosomes, 65–70% of the proteins were nonhistone proteins; this contrasts with the value, 40%, for nonhistone proteins associated with nucleosomes containing bulk DNA. (*ii*) The amount of H1 histone in chromatin containing predominantly bulk DNA was about 13.7%. However, the H1 histone was depleted and possibly absent in component α oligonucleosomes. (*iii*) Coincident with the decrease in the H1 histones and in the same molecular weight range (24,000–43,000), there appeared five minor nonhistone proteins. The minor, low-molecular-weight, nonhistone proteins were not detected in chromatin containing bulk DNA but they represented nearly 12% of the protein in component α nucleosomes. The resistance to salt extraction (0.6–2.0 M NaCl) indicates that the low-molecular-weight nonhistone proteins are tenaciously bound to the component α nucleosomes. In addition, a class of high-molecular-weight (>100,000) nonhistone proteins was enriched 5- or 6-fold in component α oligonucleosomes. The relative amounts of the nucleosome core histones were not changed.

Eukaryotic chromatin is organized into arrays of subunits or nucleosomes. Each nucleosome contains 180 to 200 base pairs of DNA in association with an octameric histone complex of two of each of the histones H2a, H2b, H3, and H4 (1).

We have explored the relationship between the subunit structure of chromatin and the organization of highly repetitive DNA sequences in mammalian cells, using site-specific restriction endonucleases and micrococcal nuclease (2–5). It was observed that the highly repetitive component α DNA of the African green monkey contains cleavage sites for *Eco*RI endonuclease with a periodicity (176 ± 4 nucleotide base pairs) approximating the spacing of the initial sites for micrococcal nuclease attack in the total chromatin (3). The results of digestions of monkey nuclei with restriction endonucleases are consistent with a specific register of the component α DNA repeat sequence within the nucleosome structure of monkey chromatin. In the observed register, the *Eco*RI cleavage sites in component α DNA are located in the interstices between adjacent nucleosomes where they are accessible to attack by added *Eco*RI (5). On the basis of these observations, a method has been developed for the isolation of intact polynucleosomal arrays containing only the component α DNA.

The possibility of preparing and fractionating intact deoxyribonucleoprotein structures containing a specific repetitive DNA sequence allows a detailed study of the nucleosomal organization of eukaryotic chromatin. Several questions may now be resolved. For example, are there structural and compositional properties that distinguish component α nucleosomes from those monkey nucleosomes that do not contain highly repetitive DNA? Are there aspects of the nucleosomal organization that may explain the apparent alignment of the repeat sequence of component α DNA within the nucleosome structure (5)? Could such differences account for the transcriptional inertness of this repetitive DNA or the constitutively heterochromatic nature of its chromatin? In this preliminary report, a method for isolating specific nucleosomal arrays is described and some results of their compositional analyses are presented and discussed.

MATERIALS AND METHODS

Cell Culture and Preparation of Nuclei. CV-1 cells, an established heteroploid cell line derived from African green monkey (*Cercopithecus aethiops*) kidney cells, were grown as monolayers in Eagle's minimal essential medium supplemented with nonessential amino acids, 5% (vol/vol) calf serum, and 5% (vol/vol) fetal calf serum. The cells were harvested and the nuclei were prepared as described (3). Before enzyme treatment, the nuclei were washed once in the *Eco*RI restriction buffer (100 mM Tris·HCl, pH 7.4/50 mM NaCl/5 mM $MgCl_2$/0.02% Nonidet P-40).

Nuclease Digestion of Isolated Nuclei and Analysis of Products. *Eco*RI was prepared from *Escherichia coli* strain RY-13 according to the method of Greene *et al.* (6). Micrococcal nuclease was obtained from Worthington Biochemicals Corp., and protease K from E.M. Biochemicals. For digestion with *Eco*RI endonuclease, the washed CV-1 nuclei were resuspended in *Eco*RI buffer at 5×10^7 nuclei per ml and incubated with the restriction enzyme, 1000 units/ml, at 37° for 2.5 hr. For digestion with micrococcal nuclease, the nuclei were resus-

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Abbreviation: $NaDodSO_4$, sodium dodecyl sulfate.

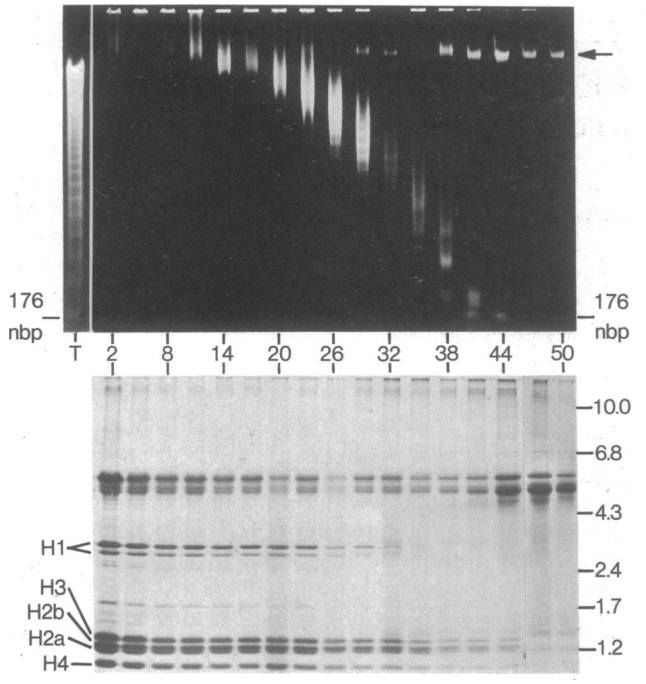

FIG. 1. Gel electrophoresis patterns of the DNA and proteins of nucleosomes released by *Eco*RI digestion of CV-1 nuclei and fractionated on isokinetic sucrose gradients. Isolated CV-1 nuclei (5×10^7/ml) in *Eco*RI restriction buffer were digested with *Eco*RI endonuclease (1000 units/ml) at 37° for 2.5 hr. The digested nuclei were sedimented and the nucleosomes were released by resuspension in 0.5 mM EDTA, pH 7.0. Aliquots of the nucleosome suspension were layered onto isokinetic sucrose gradients and centrifuged at 22,000 rpm for 18 hr at 4° in a Beckman SW 27 rotor. The gradients were buffered with 2 mM Tris·HCl, pH 7.0/1 mM EDTA and had the following parameters: c_t = 5% sucrose; c_r = 28.8% sucrose; v_m = 33 ml (7). The direction of sedimentation is from right to left in the figure. The gradients were collected from the bottom into 51 fractions of 0.75 ml each. To reduce the number of samples, fractions were pooled in consecutive groups of three. The fraction numbers, shown between the panels, refer to the middle fraction of each pool (that is, number 38 represents the pool of fractions 37, 38, and 39). DNA and protein were prepared for electrophoresis as described in *Materials and Methods*. Migration was from top to bottom in all gels shown. The gradient is divided into two regions: region I (fractions 2–29) contains mostly main band DNA chromatin, and region II (fractions 32–44) consists of component α nucleosomes. nbp = nucleotide base pairs.

(*Upper*) Photograph taken in ultraviolet light after electrophoresis in a 1.4% agarose gel. Lane T contained DNA extracted from the total nucleosome suspension before fractionation. The arrow on the right indicates high-molecular-weight DNA near the top of the gel (see *text*) and 176 nbp refers to the monomeric length of component α *Eco*RI segments.

(*Lower*) Nucleosomal proteins from sucrose gradient fractions. The proteins were electrophoresed in $NaDodSO_4$/polyacrylamide gels and stained with Coomassie blue. Each protein sample is aligned with the corresponding agarose gel pattern of the DNA (*upper*) from the same pooled fractions of the sucrose gradient. The numbers to the right of the photograph refer to the size of the proteins in daltons $\times 10^{-4}$.

The amount of 50,000–60,000 dalton proteins, although relatively constant (19–22%) throughout regions I and II, was appreciably increased in fractions 44 (containing α mononucleosomes) and 47–50, which do not contain DNA. Perhaps the increase in this class of proteins in fraction 44 results from the sedimentation of unbound nucleosomal proteins into the top of the gradient. When the centrifugation time was increased, the mono- and oligonucleosomes sedimented further into the gradient and displayed a protein pattern in $NaDodSO_4$ gels like that shown here for fractions 32–41. Under these conditions, intact nucleosomal arrays were well separated from the free proteins which remained near the top of the gradient.

pended in *Eco*RI buffer supplemented with 0.25 mM $CaCl_2$; the micrococcal nuclease concentrations varied from 45 units/ml for 5 min to 450 units/ml for 10 min, at 37°. All enzymatic digestions were terminated by chilling and centrifuging the incubation mixtures at $3000 \times g$ for 10 min at 4 °. The pelleted chromatin was lysed by resuspension in 0.5 mM EDTA, pH 7.0. Insoluble material was removed by centrifugation for 2 min at $3000 \times g$.

The nucleosome arrays released from the nuclei by either *Eco*RI or micrococcal nuclease were fractionated by velocity sedimentation through isokinetic sucrose gradients (7). For DNA analysis, aliquots from sucrose gradient fractions were deproteinized by digestion with protease K at 50 μg/ml containing 1% (wt/vol) sodium dodecyl sulfate ($NaDodSO_4$), at 37° for 4–6 hr. The DNA was precipitated with ethanol, dissolved, and analyzed by agarose gel electrophoresis (3). The proteins from the same fractions were precipitated with 20% (vol/vol) trichloroacetic acid, washed with acetone, and then analyzed by $NaDodSO_4$/polyacrylamide gel electrophoresis (8). The relative amounts of protein in individual bands or groups of bands were calculated from areas under the curves of microdensitometer tracings of negatives obtained from photographs of gels stained with Coomassie blue.

Test for Component α DNA. To distinguish whether component α or main band DNA was present in individual fractions from a sucrose gradient, the sample DNA was denatured and reassociated at low C_0t. The reassociated DNA was analyzed by centrifugation to equilibrium in analytical CsCl buoyant density gradients (9).

RESULTS

Preparation of Component α Nucleosomes: The Method. *Eco*RI endonuclease specifically cleaves component α DNA in intact nuclei of monkey cells (3, 5). Because the monkey main band DNA contains few and widely spaced sites for *Eco*RI (3), this cleavage excises and releases oligonucleosomal arrays containing only component α DNA (component α nucleosomes). The excised component α chromatin is a collection of polynucleosomes differing in length as integral multiples of the unit nucleosome and can be fractionated by isokinetic sucrose gradient centrifugation. The specific *Eco*RI attack decreases the molecular weight of the component α chromatin so that it can be separated from the high-molecular-weight bulk DNA chromatin. Thus, nonspecific nucleases and harsh treatments such as sonication or high-speed shearing are avoided; these mechanical treatments, essential parts of other commonly used methods of chromatin fractionation, disrupt the native structure of chromatin (10) and induce protein migrations and exchanges (11). To prevent introducing similar artifacts in preparing and using a chromatin gel, only isolated nuclei were used as the starting material.

Fractionation of Component α Polynucleosomes. Digestion of isolated monkey nuclei with *Eco*RI endonuclease resulted in the DNA cleavage pattern illustrated in lane T of Fig. 1 *upper*. The presence of the multimeric series of segments based upon a monomer size of 176 ± 4 nucleotide base pairs indicated that component α DNA sequences within the chromatin were cleaved by the *Eco*RI endonuclease (3, 5). The released polynucleosomes were fractionated by sedimentation through isokinetic sucrose gradients. The DNAs in the sucrose gradient fractions were subjected to agarose gel electrophoresis to determine the size distribution of the fractionated polynucleosomes. As shown in Fig. 1 *upper*, the lower portion of the gradient (region I: fractions 2–29) contained the high-molecular-

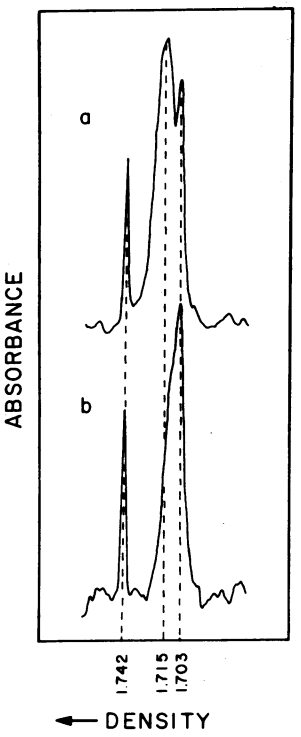

FIG. 2. CsCl buoyant density profiles of reassociated DNA of fractionated nucleosomes released from CV-1 nuclei by *Eco*RI. The sample DNA, in 10 mM Tris·HCl, pH 8.4/1 mM EDTA, was denatured at 98° for 6 min, NaCl was then added to 0.3 M, and the DNA reassociated to a C_0t of 1.0 at 60°. Under these conditions, component α DNA reassociates rapidly and forms a band at ρ = 1.703 g/ml; main band sequences reassociate poorly at low C_0t and form a diffuse band at ρ = 1.715 g/ml (9). CsCl and SP-8 standard DNA (ρ = 1.742 g/ml) were added and the mixture was centrifuged to equilibrium in analytical buoyant density gradients. (*a*) Buoyant density profile of the DNA of fraction 20 after reassociation. This fraction was obtained from region I of the sucrose gradient (Fig. 1 *upper*). (*b*) Buoyant density profile of the DNA of fraction 32 after reassociation. This fraction was obtained from region II of the sucrose gradient (Fig. 1 *upper*). A shoulder at ρ = 1.709 g/ml is also present in the microdensitometer tracing: this density is too low for main band DNA sequences. The shoulder seems to be due to structures of high electrophoretic mobility formed during the reassociation of short restriction segments of component α DNA. These structures, which are not formed during the reassociation of high-molecular-weight DNA, have been described previously (3).

weight main band DNA chromatin and component α nucleosomal arrays longer than the decapentanucleosome. The upper portion of the sucrose gradient (region II: fractions 32–44) contained component α oligonucleosomes ranging from the decapentanucleosome to the mononucleosome. High-molecular-weight component α DNA was also present in region II as shown at the arrow. The slow sedimentation rate of this high-molecular-weight DNA suggested that it may exist in an extended form with little associated protein.

The extent of contamination of region II with chromatin containing main band DNA was measured by denaturing and reassociating the sample DNA before banding it in analytical CsCl buoyant density gradients. Fig. 2 *upper* shows that the DNA in fraction 20 (region I) of the sucrose gradient is 76% main band and 24% component α DNA. In contrast, the DNA of gradient fraction 32 (region II) is component α as shown by the band at ρ = 1.703 g/ml characteristic of reassociated component α (Fig. 2 *lower*). Thus, the lack of any detectable band at a density of 1.715 g/ml and the presence of a major band at a density of 1.703 g/ml indicates that region II of the sucrose gradient contained component α nucleosomes without significant contamination with main band DNA chromatin.

A method similar to that described here has recently been used to isolate nucleosomes containing the satellite I DNA sequences of the calf (12). The protein composition of the nucleosomes containing this satellite DNA was not reported.

Nucleosomal Proteins. The protein composition of the fractionated component α nucleosomes was analyzed by electrophoresis in $NaDodSO_4$/polyacrylamide gels. The gel patterns in Fig. 1 *lower* show that the types and amounts of proteins varied in different nucleosomal fractions of the sucrose gradient.

(*i*) In region I, nonhistone proteins accounted for about 40% of the total protein associated with polynucleosomes containing mostly bulk DNA. About one-half of these nonhistone proteins (about 22% of the total protein) migrated as if they were between 50,000 and 60,000 daltons. It is possible that some of these 50,000–60,000 dalton proteins represent nuclear matrix proteins although they were present in too large an amount (>22%) to represent only matrix proteins [about 12% (13)].

(*ii*) In gradient fractions containing only component α nucleosomes (region II), 65–70% of the proteins were nonhistones (e.g., fraction 38). This increase in relative nonhistone protein content results from a decrease in the amount of total histone (depletion of H1 histone) in addition to increases in the amounts of some low- and high-molecular-weight classes of nonhistone proteins (see below). Therefore, the amount of nonhistone protein contained in monkey nucleosomes ranges from 40% (bulk DNA) to 70% (component α) of the total nucleosomal protein. This is a significantly greater amount of nucleosomal nonhistone protein than the values reported for isolated chick erythrocyte nucleosomes [6% (14)] or rat liver nucleosomes [20% (15)].

(*iii*) As the length of the component α nucleosomal arrays decreased to the mononucleosome size, the protein pattern also changed. This was particularly evident in two regions of the protein gel pattern:

(*a*) In the gel region representing >100,000 daltons, high-molecular-weight proteins were present throughout the gradient (about 4.5% of the total in fraction 17). However, these proteins were greatly enriched in the component α nucleosomes of region II (about 24% of the total in fraction 38).

(*b*) In the region of histone H1 (24,000–43,000 daltons), bands also displayed marked alterations. Most obvious was the depletion and possible elimination of the two pronounced histone H1 bands in the component α nucleosomes (Figs. 1 *lower* and 3). Coincident with this change and in this same molecular weight region there appeared, with increasing intensity, five minor protein bands. The proportion of the nucleosomal protein represented by these five bands approached 12% of the protein in fraction 38. The relative amounts of histones H2a, H2b, H3, and H4 were not changed in the component α nucleosomes.

Properties of the Low-Molecular-Weight Proteins. Two of the 24,000–43,000 dalton minor protein bands had mobilities similar to those of the two histone H1 bands. The similar mobilities made it difficult to determine whether the H1 histones were completely absent from the component α nucleosomes in the range from the pentanucleosome to the mononucleosome. To test whether these minor bands represented nonhistone proteins or modified forms of histone H1, we extracted the nucleoproteins of isolated CV-1 nuclei selectively with 0.6 M NaCl (16). This treatment solubilized many nonhistone proteins in addition to the H1 histones (Fig. 4). However, comparison of Figs. 3 and 4*c* shows that the insoluble proteins contained five proteins with electrophoretic mobilities

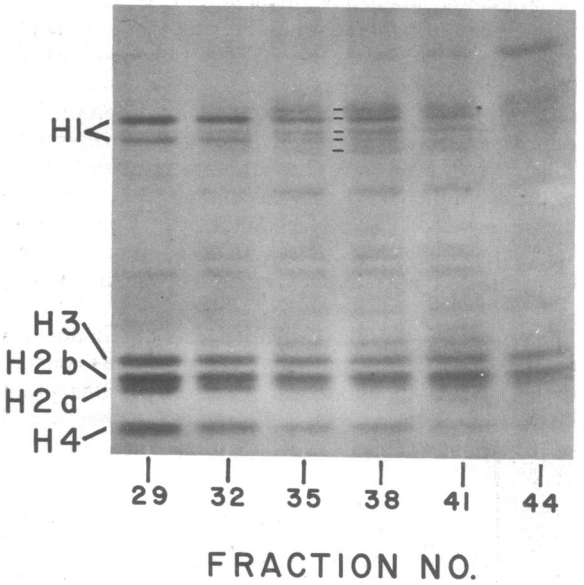

FIG. 3. Change in the gel pattern of the low-molecular-weight nucleosomal proteins of region II. This photograph is an enlargement of the protein gels of fractions 29–44 shown in Fig. 1 *lower* and encompasses only the region of 12,000–50,000 daltons. The five low-molecular-weight nonhistone proteins are indicated by the lines between the gel patterns of fractions 35 and 38.

apparently identical to those of the minor bands seen in the component α nucleosomes. The minor protein bands were also found in the insoluble pellet after extraction of CV-1 nuclei with 2.0 M NaCl or 0.2 M H_2SO_4.* Both of these reagents remove the histone proteins. The results indicate that the low-molecular-weight protein bands represented neither modified H1 histones nor other types of histones; rather, they are nonhistone proteins.

Histones not bound to chromatin show a greater susceptibility to proteolytic attack than do bound histones (17). Possibly, the absence of the H1 histone in component α oligonucleosomes was due to a protease activity in the *Eco*RI endonuclease preparation. The incubation of component α chromatin in the presence of the *Eco*RI endonuclease may have rendered histone H1 more accessible to proteolysis. However, several experiments indicated that this hypothesis is not correct.

When H1 histone was salt-extracted from CV-1 nuclei and incubated with large amounts of the *Eco*RI endonuclease, no degradation of histone H1 or of any of the other solubilized nucleoproteins was observed.* To test whether another component in the *Eco*RI preparation removed the H1 histone from the chromatin, isolated CV-1 nuclei were incubated for 3 hr with the *Eco*RI preparation in restriction buffer lacking $MgCl_2$. The absence of $MgCl_2$ prevented the cleavage of the component α nucleosomes. $CaCl_2$ was then added (0.25 mM) and the nuclei were digested with micrococcal nuclease. In a control experiment, nucleosomes were also prepared by micrococcal nuclease digestion without prior incubation with the *Eco*RI endonuclease. The nucleosomes from the two digests were fractionated on isokinetic sucrose gradients and the protein gel patterns of the various fractions were compared. No differences in the protein patterns were detected in fractions from equivalent regions of the two sucrose gradients. The relative amounts of histone H1 were not depleted in any of the nucleosome fractions by prior incubation of nuclei with the *Eco*RI preparation and no enrichment in the minor, low-molecular-weight nonhistone

* P. R. Musich, F. L. Brown, and J. J. Maio, unpublished data.

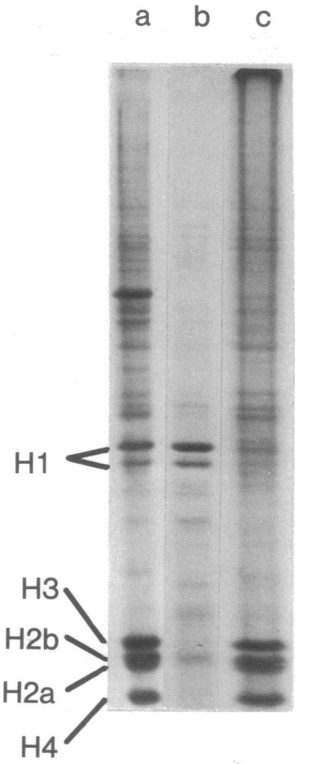

FIG. 4. Gel electrophoretic patterns of CV-1 nuclear proteins after extraction of the H1 histone. Isolated CV-1 nuclei were extracted by resuspending them in 10 mM Tris·HCl, pH 8.0/5 mM $Na_2S_2O_5$/0.6 M NaCl (16), mixing for 15 min at 4°, and centrifuging for 19 hr at 4° in a Beckman SW 50.1 rotor at 45,000 rpm. The proteins in the pellet and supernatant fractions were analyzed by $NaDodSO_4$/polyacrylamide gel electrophoresis (8). (*a*) Total nuclear proteins; (*b*) nuclear proteins soluble in 0.6 M NaCl (supernatant fraction); (*c*) nuclear proteins not soluble in 0.6 M NaCl (pellet fraction).

bands was detected.* Thus, the deficiency of the H1 histones in the component α oligonucleosomes does not seem to be an artifact of the *Eco*RI digestion conditions.

The question arises whether the enrichment of the small (24,000–43,000 daltons) and large (>100,000 daltons) nonhistone proteins in nucleosomes of region II reflects specific protein associations with component α DNA or properties of any short nucleosomal arrays sedimenting in this part of the gradient. To answer this question, a population of nucleosomes representative of the total genome was generated by micrococcal nuclease digestion of CV-1 nuclei in *Eco*RI buffer. A similar digest was prepared in the low phosphate/$CaCl_2$ buffer described previously (3). The nucleosomes were fractionated on isokinetic sucrose gradients and the protein and DNA from the gradient fractions were analyzed. Regardless of the length of the polynucleosomes released by micrococcal nuclease, no enrichment was observed for either the large or small nonhistone proteins (Fig. 5). In addition, no significant depletion in histone H1 was observed in the oligonucleosome fractions. These results were independent of whether the CV-1 nuclei were partially (10% monomers) or extensively (70% monomers) digested with micrococcal nuclease. They were also independent of whether the micrococcal nuclease digestion was in *Eco*RI buffer or in the standard low phosphate/$CaCl_2$ buffer.

DISCUSSION

Three basic observations suggest an altered configuration of the protein–DNA complexes in component α chromatin: (*1*) the

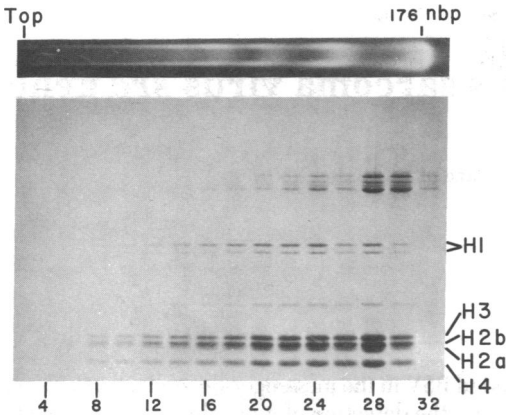

FIG. 5. Gel electrophoresis patterns of the DNA and proteins of nucleosomes released by micrococcal nuclease digestion of CV-1 nuclei and fractionated on isokinetic sucrose gradients. Isolated CV-1 nuclei (5×10^7/ml) in *Eco*RI buffer supplemented with 0.25 mM $CaCl_2$ were digested with micrococcal nuclease (180 units/ml) at 37° for 10 min. The digested nuclei were sedimented and the nucleosomes were released by resuspension in 0.5 mM EDTA, pH 7.0. The proteins were electrophoresed in $NaDodSO_4$/polyacrylamide gels and stained with Coomassie blue. Migration was from left to right in *Upper* and from top to bottom in *Lower*. The numbers at the bottom refer to fractions of the sucrose gradient. (*Upper*) DNA was purified from a portion of the nucleosomes by deproteinization and analyzed by 1.4% agarose gel electrophoresis. About 30% of the DNA in this digest migrated as monomer fragments [185 nbp (3)] or smaller. The remaining portion of the nucleosomes was fractionated in isokinetic sucrose gradients as described in Fig. 2. The gradients were collected from the bottom into 34 fractions of about 1.1 ml each. For analysis, the fractions were pooled in consecutive groups of two. (*Lower*) Proteins from the sucrose gradient fractionated nucleosomes. Fractions 26–30 contained the mononucleosomes, with the peak of the distribution in fraction 28.

absence of histone H1 within short component α oligonucleosomes; (*ii*) the appearance of certain classes of nonhistone proteins in the H1 histone-deficient component α oligonucleosomes; and (*iii*) a tenacious binding of the low-molecular-weight nonhistone proteins to the component α chromatin. These observations are consistent with the interpretation that certain nonhistones may be specifically associated with the component α nucleosomes. It is notable that some of these proteins migrated with an electrophoretic mobility similar to that of H1 histone and became enriched in the component α oligonucleosomes where the H1 histones were depleted. In addition, component α nucleosomes showed a 5- or 6-fold enrichment in a series of large (>100,000 daltons) proteins of unknown nature. In the high-molecular-weight chromatin, containing mostly main band DNA, the H1 histone constituted about 13.7% of the nucleosomal protein (Fig. 1 *lower*, fraction 17). On the other hand, the low-molecular-weight nonhistone proteins represented nearly 12% of the total protein in component α nucleosomes (Figs. 1 *lower* and 3, fraction 38). This similarity in the proportional content of these two types of proteins in different nucleosomal arrays suggests that the two classes of proteins may perform similar functions in the organization of their respective nucleosomal arrays. However, they may have quite different consequences for transcription.

The chromatin containing satellite and highly repetitive DNA sequences is less susceptible to nuclease attack than is the remainder of the nuclear chromatin (18). In studies of the subnuclear distribution of mouse satellite DNA it was observed that some nonhistone proteins were tenaciously bound to the satellite DNA (19). It is possible that such tightly bound proteins determine the highly condensed, heterochromatic organization of the chromatin containing the mouse satellite sequences and render this chromatin less susceptible to cleavage by nuclease (P. R. Musich, K. W. C. Peden, and R. S. Reis, unpublished data). In the studies presented here, the low-molecular-weight nonhistone proteins of component α nucleosomes of the African green monkey also resisted salt extraction (0.6–2.0 M NaCl). Preliminary results indicate a similar enrichment of certain tightly bound, low-molecular-weight, nonhistone proteins and a depletion of the H1 histone in calf satellite I chromatin prepared as described here (unpublished data).

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