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Comparison of the Folding of β-Globin and Ovalbumin Gene Containing Chromatin Isolated from Chicken Oviduct and Erythrocytes

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ABSTRACT: The dependence of chromatin conformation upon salt concentration has been studied for chicken ovalbumin and β-globin genes isolated from oviduct and adult erythrocytes. At NaCl concentrations of 25 or 50 mM, the sedimentation properties, as a function of DNA size, of ovalbumin and globin chromatin are similar regardless of the source of the chromatin. In 100 mM NaCl, however, β-globin chromatin isolated from erythrocytes sediments more slowly than an ovalbumin chromatin fraction from erythrocytes containing DNA of the same size. When the same experiment is carried out with material isolated from oviduct nuclei, the relative sedimentation rates are reversed, so that the ovalbumin chromatin sediments more slowly. This behavior cannot be accounted for by differences in binding of RNA polymerase or other molecules associated with transcription, or by partial aggregation of the chromatin. The most reasonable explanation is that transcriptionally active chromatin with a history of transcriptional activity, although largely covered with histones and capable of considerable compaction, is not able to form a fully compact structure as the ionic strength is raised. This behavior is consistent with a slight depletion in active chromatin of core histones or histone H1/H5 or both.

The chromatin of genes that are expressed is distinguished from bulk chromatin by an unusually sensitivity to nucleases. On the other hand, at least in the case of genes that are transcribed at only moderate rates, a considerable fraction of the transcribed sequence can be found packaged in nucleosomes. It is possible that these facts could be explained by the partial disruption of the 30-nm-thick chromatin fiber in the neighborhood of transcriptionally active genes, presumably resulting from partial loss of histone H1 or of core histone octamers, or from the unfolding of the octamers after some modification [see, for example, Karpov et al. (1984)].

In earlier studies, the folding of chromatin into 30-nm fibers was examined by using sequence-specific probes that permitted comparison of expressed with inactive genes. Felsenfeld et al. (1982) found that at concentrations of NaCl up to 50 mM, the sedimentation properties of chromatin fibers from chicken erythrocytes containing the adult β (β^A) globin gene were indistinguishable from those of bulk chromatin. On the other hand, Kimura et al. (1983), using a chromatin fraction excised

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from nuclei by digestion with endonuclease *Eco*RI, observed that in 80 mM NaCl, β^A-globin chromatin sedimented more slowly than chromatin fragments of the same size containing inactive genes. This suggested that this globin chromatin fraction was incapable of complete folding.

In this paper, we describe experiments which measure the sedimentation properties of both the β^A-globin and ovalbumin genes contained in chromatin prepared from both chicken erythrocytes and oviduct. We find that in 25 or 50 mM NaCl the two genes behave similarly when isolated from either tissue, but in 100 mM NaCl chromatin fragments containing the active gene sediment more slowly than those of the same size containing the inactive gene.

The results suggest that although polynucleosome filaments containing expressed genes are capable of partial compaction, they are not able to form a completely ordered structure.

Materials and Methods

Chromatin. In the preparation of oviduct nuclei, two to four adult actively laying white leghorn hens were used per experiment. [Adult laying hens have higher relative and absolute amounts of ovalbumin mRNA than do steroid-induced chicks (Shutz et al., 1977).] The animals were euthanized by an overdose of pentobarbital injected into a wing vein and immediately dissected. The foramen magnum portions of the oviducts were removed and placed in a glass tray containing 500 mL of ice-cold phosphate-buffered saline modified by the addition of phenylmethanesulfonyl fluoride (PMSF) and sodium butyrate to 0.1 and 5 mM, respectively. The foramen magnum portions were cut open to flatten them, and blood clots were removed by gentle scraping with a single-edge razor blade. The tissue was placed in a tray with 250 mL of ice-cold fresh modified phosphate-buffered saline solution, and more vigorous scraping was performed to remove the parenchymal cells from the connective tissue support. The cells were centrifuged and washed twice in 160 mL of the modified phosphate-buffered saline at 4 °C and 2000 rpm for 5 min in an HB-4 Sorvall rotor. Subsequent operations were carried out at 0–4 °C. The cells were resuspended in 160 mL of buffer containing 50 mM NaCl, 10 mM tris(hydroxymethyl)-aminomethane hydrochloride (Tris·HCl), pH 8, 2.5 mM $MgCl_2$, 0.25% Triton X-100, 0.3 M sucrose, 5 mM sodium butyrate, and 0.1 mM PMSF. The cells were then homogenized by three strokes in a Thomas glass vessel (size C) using a motor-driven Teflon serrated pestle. The homogenate was filtered through four layers of gauze and centrifuged at 2000 rpm for 5 min in the HB-4 rotor. The nuclear pellet was resuspended and washed twice in 160 mL of buffer containing 50 mM NaCl, 10 mM Tris·HCl, pH 7.5, 3 mM $MgCl_2$, 5 mM sodium butyrate, and 0.1 mM PMSF (modified RSB). The nuclei were resuspended in 80 mL of a buffer containing 10 mM Tris·HCl, pH 8.0, 2.5 mM $MgCl_2$, 0.1% Triton X-100, 50 mM NaCl, 5 mM sodium butyrate, 0.1 mM PMSF, and 1.2 M sucrose. This suspension was centrifuged at 4000 rpm for 30 min in the HB-4 rotor.

The nuclear pellets were resuspended at an A_{260} of 50 with modified RSB. $CaCl_2$ was added to 0.5 mM, and the nuclei were digested with micrococcal nuclease (Worthington) at 15–18 units/mg of DNA for 20 min at 4 °C. The digestion was stopped by the addition of ethylenediaminetetraacetic acid (EDTA) and ethylene glycol bis(β-aminoethyl ether)-*N*,*N*,-*N*′,*N*′-tetraacetic acid (EGTA) (to 2.5 mM each), and chromatin was released by overnight dialysis at 4 °C against 1 mM EDTA, pH 8.0. Nuclear membranes were removed by centrifugation at 4 °C and 10000 rpm for 15 min in the HB-4 rotor. The optical density of the resulting supernate was measured, and 600 μg of chromatin was loaded onto isokinetic sucrose gradients containing 25, 50, or 100 mM NaCl (McCarty et al., 1974), 5 mM Tris·HCl, pH 8, and 0.1 mM EDTA. Some samples were treated with RNase T1 (Bethesda Research Laboratories; 2000 units at 37 °C for 30 min) before being loaded onto the gradients. The gradients were centrifuged in an SW40 rotor (Beckman) at 4 °C and 35000 rpm for 120–180 min. Fractions were collected, and the optical density of each fraction at 260 nm was measured. The fractions were treated with proteinase K (Beckman) at 100 μg/mL and sodium dodecyl sulfate ($NaDodSO_4$) at 0.1% and then extracted twice each with equal volumes of phenol/chloroform/1 M Tris (9:10:1) and chloroform. The resulting aqueous phase was made 100 mM in NaCl and precipitated with an equal volume of 2-propanol at room temperature. After centrifugation, the pellets were washed twice with 70% ethanol and briefly vacuum dried.

Erythrocyte nuclei were obtained from adult chicken blood. The red blood cells were collected by centrifugation for 5 min at 4 °C and 2000 rpm in the HB-4 rotor and thereafter processed in a fashion similar to that for the oviduct homogenate. Red cells were pelleted from adult chicken blood, washed twice in modified phosphate-buffered saline, and then resuspended in buffer containing 50 mM NaCl, 10 mM Tris·HCl, pH 8, 3 mM $MgCl_2$, 0.25% Triton X-100, 5 mM sodium butyrate, and 0.1 mM PMSF. Nuclei released by this treatment were washed twice in this buffer and then resuspended and in some cases washed twice in a buffer of the same ionic composition, but with Triton omitted, before sedimentation through the latter buffer which had been made 1.2 M in sucrose. In the case of the preparation used for the experiments in Figures 3 and 4, this buffer also contained 0.1% Triton. The concentration of $CaCl_2$ was adjusted to between 0.5 and 2 mM, and the nuclei were digested with 140–180 units of micrococcal nuclease per milligram of DNA. Precise digestion conditions for each preparation were determined by analysis of DNA size distribution obtained from pilot digests.

Electrophoresis and Blotting. The DNA pellets were resuspended in 50 μL of 5 mM Tris·HCl, pH 8, and 0.1 mM EDTA, pH 8. In most cases, 5 μg of DNA from each fraction as well as 5 μg from the unfractionated chromatin was electrophoresed in a 0.8% agarose gel in a buffer containing 0.04 M Tris, 0.02 M acetic acid, and 1 mM EDTA. Electrophoresis was carried out for 4 h at 100 V. The gels were stained in 0.1% ethidium bromide, photographed, and then transferred overnight onto a Gene Screen Plus membrane (New England Nuclear) in 1.5 M NaCl/0.15 M sodium citrate (10× SSC) using the protocol supplied by the manufacturer.

The membranes were sealed in plastic bags containing the alternate method buffer (no formaldehyde) given in the New England Nuclear protocol and placed in a shaking 65 °C water bath overnight. Hybridization probes were labeled with ^{32}P by nick translation. The probe for the β-globin gene was the plasmid pCAβG1 [described in McGhee et al. (1981)] and for the ovalbumin gene, the plasmid pOV2.4 [provided by Dr. B. O'Malley and described in Woo et al. (1981)]. Hybridization was performed overnight at 65 °C in a shaking water bath after the addition to the bags of (3–5) × 10^6 cpm of the ^{32}P-labeled denatured probe DNA. After hybridization, the filters were washed 3 times for 5 min at room temperature in 2× SSC and then twice at 65 °C (for the globin probe) or 52 °C (for the ovalbumin probe) in 0.1 × SSC and 1% $NaDodSO_4$. The filters were blotted dry and exposed to Kodak XAR film with DuPont Lightening Plus intensifying screens at −70 °C. Autoradiograms were scanned with a Joyce-Loebl

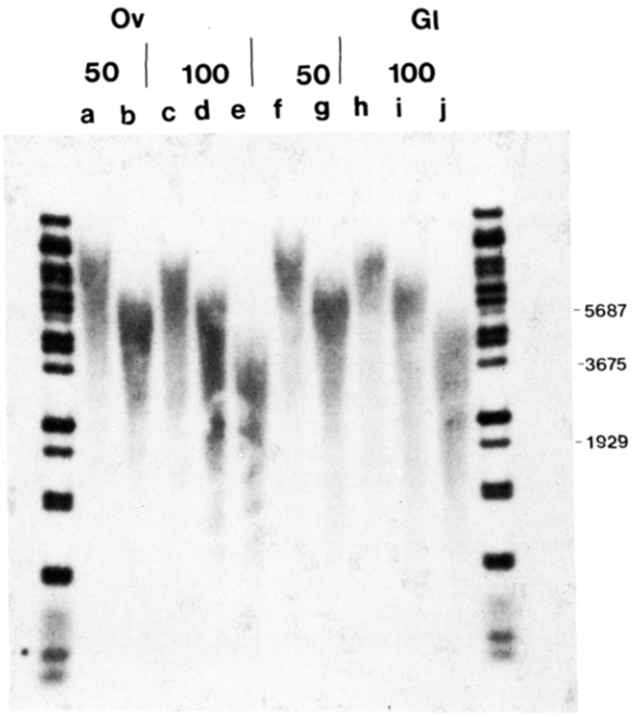

FIGURE 1: Southern blot probed with ovalbumin-specific DNA (lanes a–e) or with β-globin-specific DNA (lanes f–j). Samples were taken from peak sucrose gradient fractions of adult erythrocyte chromatin (Materials and Methods) run in 50 mM NaCl (a, b, f, g) or 100 mM NaCl (c–e and h–j). Identical pairs of samples were run on one gel in lanes (a, f), (b, g), (c, h), (d, i), and (e, j). After blotting, the Gene Screen Plus membrane was cut between lanes e and f, and the two halves were hybridized separately as described above. ^{32}P-Labeled markers (a mixture of digests of λ-phage DNA with *Bst*E II and *Bgl*II) are shown in the outer lanes. The sizes of some of the fragments are given at the right.

or Hoefer densitometer. The weight averages for each lane were calculated by numerical integration.

To assess the relative abundance of β-globin and ovalbumin sequences in the released chromatin, separate dot-blot experiments were performed. Nuclei were lysed by proteinase K/NaDodSO$_4$ treatment, and the DNA was purified and precipitated as described earlier. Another aliquot was digested with micrococcal nuclease and dialyzed overnight aganst 1 mM EDTA, pH 8 at 4 °C. The membranes were removed by centrifugation, and the DNA was purified and precipitated. The DNA from both aliquots was resuspended in 250 μL of 5 mM Tris·HCl, pH 8, and 0.1 mM EDTA, pH 8, and made 0.2 M in sodium hydroxide. After incubation at 37 °C for 2 h, the base was neutralized by making the samples 0.2 M in hydrochloric acid. The samples were then diluted with an equal volume of 20× SSC, and volumes containing 0.5, 1, 2, 2.5, and 3 μg of DNA were applied to a Gene Screen Plus membrane using a dot-blotting manifold. The membrane was treated as described above. The signals of the resulting autoradiogram were determined by densitometry.

DNA Binding to Chromatin. To determine the extent to which relatively protein-free DNA can either adsorb to chromatin or acquire chromatin proteins during centrifugation, 5 mL of adult chicken blood was collected and processed to soluble chromatin as described above. One milligram of chromatin was loaded onto an isokinetic sucrose gradient containing 100 mM NaCl and 5 mM Tris·HCl, pH 8, and 0.1 mM EDTA, pH 8, and centrifuged in an SW40 rotor at 35000 rpm and 4 °C for 150 min. The centrifuged tube contents were collected and dialyzed against cold 1 mM Tris·HCl, pH 8, and 0.1 mM EDTA, pH 8, to remove the sucrose and then concentrated 2-fold with Ficoll 400; 150 μg (based upon A_{260}) of this material was mixed with 34 ng (25000 cpm) of linearized pBR322 that had been nick translated with [^{32}P]dCTP. The pBR322–chromatin mixture was loaded on an isokinetic sucrose gradient containing 100 mM NaCl and 5 mM Tris·HCl, pH 8, and 0.1 mM EDTA, pH 8. A second gradient was loaded with the labeled pBR322 but no chromatin. After centrifugation, 1.5-mL fractions were collected, and the A_{260} profile was measured. The radioactivity of each fraction was determined by liquid scintillation counting.

RESULTS

Chromatin samples suitable for hydrodynamic measurements were prepared by gentle treatment of oviduct or erythrocyte nuclei with micrococcal nuclease (Materials and Methods). Digestion conditions were chosen to give DNA in the size range 2–30 kilobase pairs (kbp). After the nuclei were lysed by dialysis against EDTA, the nuclear membranes were removed by low-speed centrifugation and aliquots of the supernate loaded on isokinetic sucrose gradients containing the desired concentration of NaCl (Materials and Methods). DNA was purified from individual gradient fractions, electrophoresed, transferred to Gene Screen Plus, and hybridized to appropriate radioactive probes.

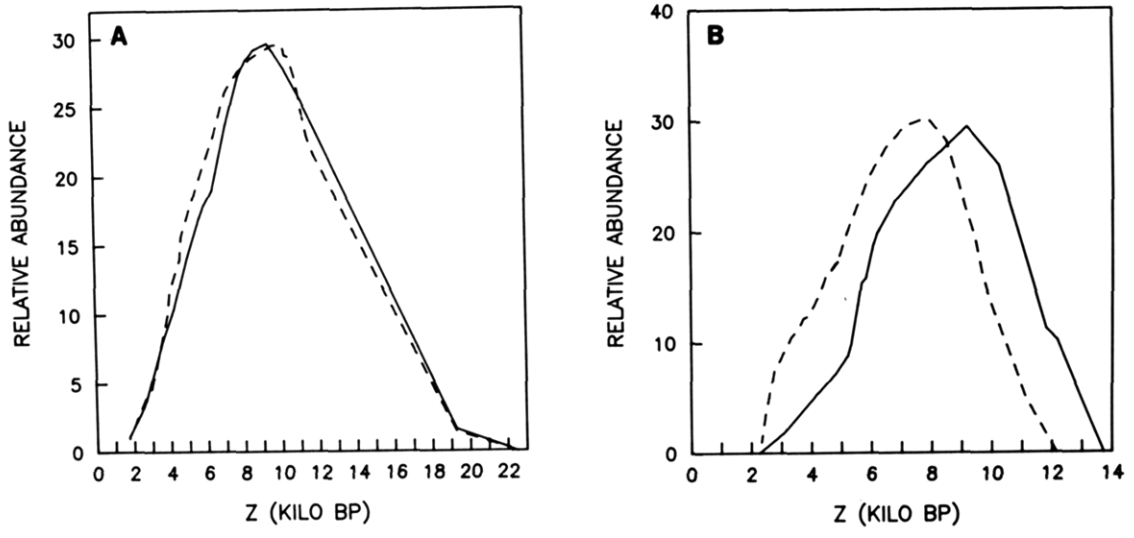

FIGURE 2: Size distribution of ovalbumin sequences (- - -) and β-globin sequences (—) in (A) lanes a and f of Figure 1 (50 mM NaCl) and (B) lanes c and h (100 mM NaCl). DNA abundance was obtained by densitometry of the autoradiogram.

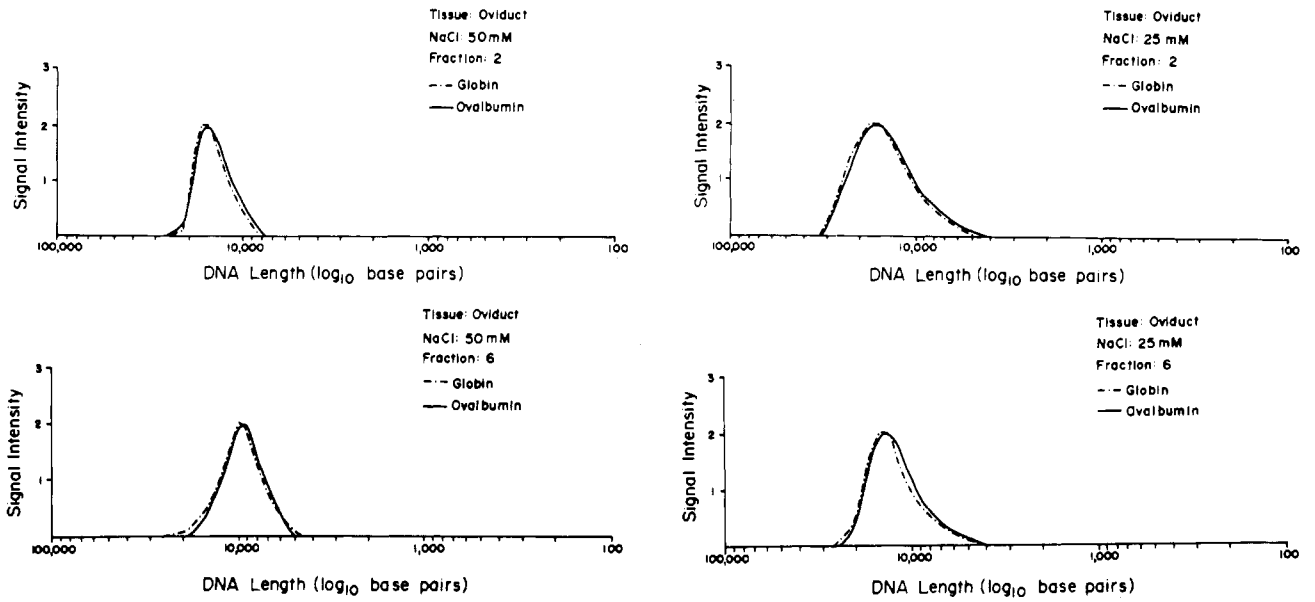

FIGURE 3: Size distribution of DNA in oviduct chromatin fractions sedimented in 50 mM NaCl (left panels) or 25 mM NaCl (right panels). Fractions isolated from the gradients were freed of protein, electrophoresed, blotted, and probed as in Figure 1 with ovalbumin- or globin-specific probes, except that the same blot was used for both probes (see Materials and Methods). DNA size distributions were obtained from autoradiograms. Size distributions in gradient fraction 4 (not shown) were intermediate between those in fractions 2 and 6 and also showed little difference between globin and ovalbumin.

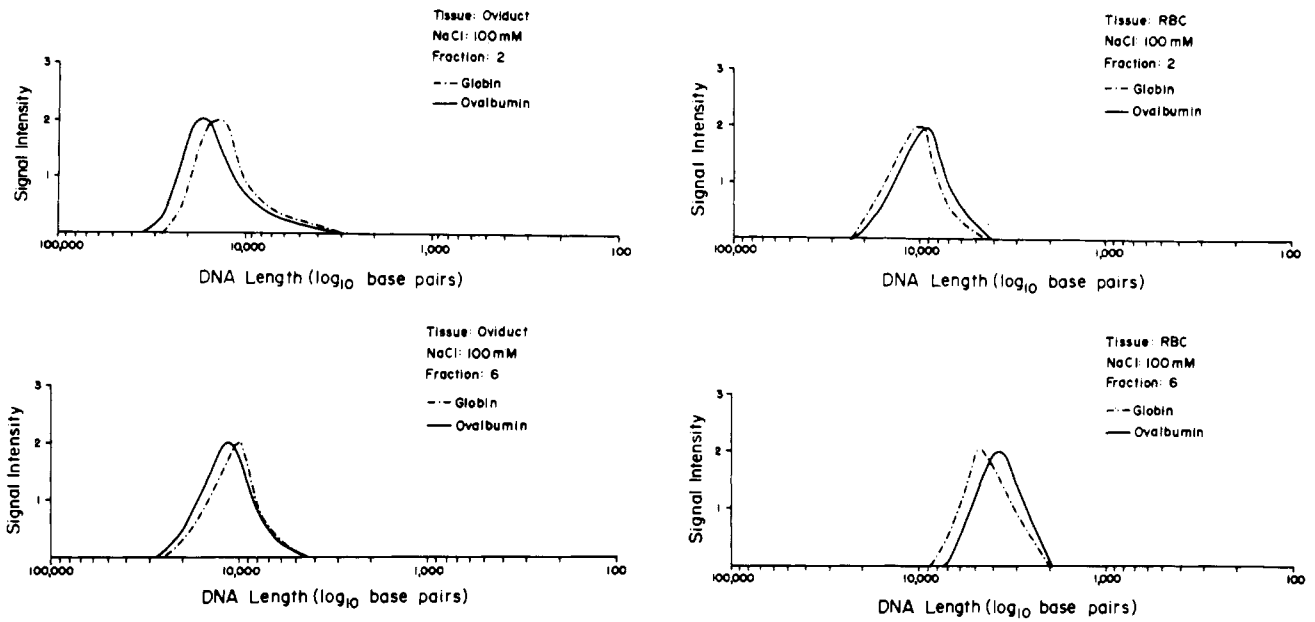

FIGURE 4: Size distribution of DNA in oviduct (left panels) or red blood cell (right panels) chromatin sedimented in 100 mM NaCl. Methods were like those given in Figure 3. Fraction 4 size distributions (not shown) were intermediate between the sizes of fractions 2 and 6 and showed similar differences between globin and ovalbumin sequences.

Results of a typical experiment with erythrocyte chromatin are shown in Figures 1 and 2. Centrifugation was carried out in the presence of 50 or 100 mM NaCl, and fractions near the peak of the sedimenting material were chosen for electrophoresis. Duplicate sets of samples were run on the same gel and, after blotting, were probed separately with either globin- or ovalbumin-specific probes. Measurement of the autoradiogram reveals that in samples from the 100 mM NaCl gradient the average size of the globin sequences is larger than in those containing ovalbumin. In the 50 mM NaCl gradients, however, the average sizes of the two sequences either are the same or differ by a smaller amount (Figure 1a; Table IB, fractions 8 and 10).

More extensive experiments were carried out with chromatin isolated from oviduct nuclei. As determined by dot blotting across the gradients (data not shown), the bulk of the globin and ovalbumin sequences was contained in the first 7 (out of a total of 10) fractions. The DNA contained in fractions 2, 4, and 6 was analyzed by electrophoresis. In these experiments, a single blot was hybridized first with the ovalbumin probe and then (after removal of this probe) with the globin probe. The autoradiograms were scanned with a microdensitometer, and the density distribution was converted to a DNA size distribution using radioactive molecular weight markers included in the blot. Results are shown in Figures 3 and 4 for fractions 2 and 6. In all experiments in 100 mM NaCl, the molecular weight distribution of the ovalbumin gene reflects higher average molecular weights in a given sucrose gradient fraction for the ovalbumin gene than for the globin gene. In 25 or 50 mM NaCl, however, only small differences are ob-

Table I: DNA Weight Averages in Gradient Fractions

	ovalbumin gene (kbp)	globin gene (kbp)
(A) Oviduct Chromatin		
25 mM NaCl		
fraction 2	15.9	16.2
fraction 4	14.5	14.8
fraction 6	13.2	13.5
50 mM NaCl		
fraction 2	14.4	14.7
fraction 4	11.6	11.8
fraction 6	9.7	10.0
100 mM NaCl		
fraction 2	15.6	13.4
fraction 4	12.0	10.5
fraction 6	11.0	9.6
(B) Erythrocyte Chromatin		
(a) 50 mM NaCl		
fraction 8	8.4	8.2
fraction 10	4.9	5.5
100 mM NaCl		
fraction 6	6.4	7.5
(b) 100 mM NaCl		
fraction 2	10.0	11.3
fraction 4	6.7	7.6
fraction 6	4.0	4.5

served. [Size distribution for fraction 4 (not shown) lead to similar conclusions.] Additional experiments with erythrocyte chromatin confirm the result shown in Figure 1. The molecular weight distributions for these erythrocyte chromatin experiments are given in Figures 2–4. The weight-average molecular weights of globin and ovalbumin sequences in each fraction are given in Table I.

In assessing the significance of these results, it is important to know what fraction of the nuclear contents is represented in the chromatin isolated from the gradients. Between 25% and 40% of the nuclear DNA is recovered in the nuclear lysate, and between 60% and 75% of the DNA in this lysate is recovered from the gradients. In the experiments with oviduct nuclei, the average size of globin sequences in the unfractionated chromatin was 17 kbp, as compared to 13 kbp for ovalbumin. Recoveries of globin and ovalbumin sequences from the gradient were comparable to the recovery of bulk chromatin. From a dot-blot assay, the abundance of globin sequences was estimated to be about 0.6 of the genomic abundance, that of ovalbumin about 1.1. There was no degradation of histones in the released chromatin as determined by polyacrylamide gel electrophoresis (data not shown).

To determine the extent to which the adsorption of relatively protein-free DNA to chromatin or the exchange of chromatin proteins onto such DNA might affect the sedimentation properties within the gradient, trace amounts of labeled, linearized pBR322 DNA were mixed with chromatin fractions from a sucrose gradient and resedimented (Materials and Methods). In the presence of chromatin, about 20% of the plasmid DNA was found at the bottom of the centrifuge tube. Of the remaining 80%, 74% migrated above the position expected for chromatin of this size (Figure 5). While much of the added DNA appears to attach itself to chromatin, the distributions on the gradient of this DNA and chromatin are quite different.

The RNA content of the oviduct chromatin described above, which was not exposed to RNase, was 3–8%. Treatment with RNase T1 (Materials and Methods) before loading on the gradients gave results similar to those shown in Figure 3.

Discussion

The 30-nm chromatin fiber undergoes reversible unfolding in solution at low ionic strength. The process has been studied

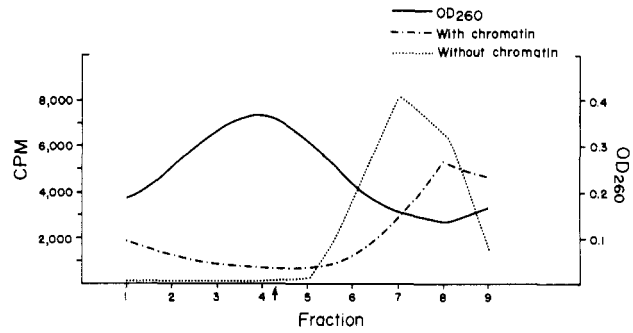

FIGURE 5: Sucrose gradient sedimentation of protein-free DNA in the presence of chromatin. This experiment measures both the adventitious binding of protein-free DNA to chromatin and the exchange of histones onto such DNA. Chromatin was isolated from adult avian erythrocytes and sedimented in 100 mM NaCl (see Materials and Methods). Fractions were pooled to collect the soluble material, and 150 μg of this chromatin was resedimented on 100 mM NaCl with 25000 cpm (34 ng) of labeled linearized pBR322. A gradient in 100 mM NaCl containing only the labeled pBR322 was also run at the same time (Materials and Methods). The absorbance profile of the chromatin-containing gradient is shown (—) as are the distributions of the linearized pBR322 in the presence (–·–) or absence (···) of chromatin. The arrow (↑) indicates the approximate expected position on this gradient of chromatin with a DNA length equal to that of linear pBR322.

by electron microscopy (Finch & Klug, 1976), by electrooptical methods (McGhee et al., 1980, 1983), by scattering of light, neutrons, and X-rays (Campbell et al., 1978; Snow et al., 1979; Hollandt et al., 1979), and by sedimentation in the ultracentrifuge (Butler & Thomas, 1980). The data show that lowering the salt concentration causes disruption of the 30-nm fiber, which is converted at low ionic strength to an extended polynucleosome filament about 10 nm in diameter. The filament refolds to form the 30-nm fiber when the salt concentration is raised.

Both the integrity of the 30-nm fiber and the ability of the 10-nm filament to refold depend upon the presence of histone H1 in normal abundance. If H1 is stripped from chromatin without disturbing the position of the core histones, it can be added back in such a way as to restore normal folding properties (Allan et al., 1981).

The most complete studies of the folding process have been carried out by Thomas and by Eisenberg and their collaborators (Butler & Thomas, 1980; Caron & Thomas, 1981; Ausio et al., 1984). Both groups have used ultracentrifugation to monitor effects of NaCl concentration on chromatin conformation. The addition of increasing amounts of salt to low ionic strength solutions results in a continuous gradual increase in the sedimentation coefficient of chromatin samples over the entire range of NaCl concentrations between 5 and 125 mM. Results from the two laboratories agree, except that in the case of rat liver chromatin, Thomas et al. observe, superimposed on the continuous increase, a small upward step in $s_{20,w}$ at 44–55 mM NaCl, which they have interpreted as reflecting an additonal stabilization of the compact structure.

In this paper, we study the salt-dependent sedimentation behavior of chromatin containing the β-globin and ovalbumin genes, isolated from both erythrocytes and oviduct. We find that there are only small differences between the size distributions of globin and ovalbumin sequences within individual sucrose gradient fractions if sedimentation is carried out in 25 or 50 mM NaCl, regardless of the source of the chromatin. [With the exception of fraction 10 (Table IB), all the differences in weight averages are 3% or less.] However, if the experiment is repeated in 100 mM NaCl, we observed a reproducible and marked difference: In chromatin from oviduct,

the average size of ovalbumin DNA in a given gradient fraction is larger than the size of β-globin DNA, while in adult erythrocyte chromatin the relative sizes are reversed. Chromatin fibers having a given length of DNA appear to sediment more slowly if they contain a gene with a history of expression. [Note that although the β-globin gene is not transcribed in adult erythrocytes, it retains the features of transcriptionally active chromatin (McGhee et al., 1981).]

Earlier results from our laboratory, obtained by using similar methods, showed that the salt-dependent sedimentation properties of β^A-globin chromatin from chick erythrocytes are indistinguishable from those of bulk inactive chromatin at NaCl concentrations up to 50 mM (Felsenfeld et al., 1982). A contrasting result was obtained by Kimura et al. (1983), who treated chicken erythrocyte nuclei with *Eco*RI restriction endonuclease to obtain chromatin fragments containing DNA of precisely defined size. These authors showed that in 80 mM NaCl, β^A-globin chromatin fragments containing 6.2 kbp of DNA migrated more slowly than chromatin containing ovalbumin and collagen gene fragments of about the same size.

These two sets of results are reconciled by the experiments reported in this paper. It appears that there is a structural anomaly in active chromatin which manifests itself only at salt concentrations greater than 50 mM, at which the normal 30-nm fiber continues to approach its completely ordered form. Our new results also suggest that this anomalous behavior is a general property of the transcriptionally active globin or ovalbumin gene and not only of the shorter, defined sequence that can be excised from the former by *Eco*RI. Furthermore, unlike the globin gene, the ovalbumin gene's coding region (Woo et al., 1981) is quite long (~7.5 kb), so that many of the chromatin fragments carrying ovalbumin gene segments will consist entirely of transcribed DNA segments. Finally, the fact that both these active genes behave similarly suggests that anomalous sedimentation at high salt concentrations may be a general property of transcriptionally active chromatin.

In trying to explain this behavior, we should not lose sight of the fact that transcriptionally active chromatin does undergo considerable compaction as the salt concentration is raised; this compaction is sufficient to make the hydrodynamic properties of active chromatin indistinguishable from bulk chromatin up to NaCl concentrations of 50 mM. This is consistent with a model in which core histone octamers and histone H1 are present in reasonable abundance. Changes in sedimentation coefficient reflect changes in shape and mass. The fact that chromatin fibers containing globin or ovalbumin genes sediment almost identically in 50 mM NaCl or lower provides further evidence that there is no large difference in histone content between the two, either in oviduct or in erythrocyte nuclei.

Certain models are eliminated by our result. It is conceivable, for example, that the binding of RNA polymerase, despite the increase in mass that is entailed, might also increase the frictional coefficient in such a way as to reduce sedimentation rates. This possibility is ruled out by the observation that in 50 mM NaCl, sedimentation rates for active and inactive chromatin are nearly identical. Similar arguments exclude an effect of nascent RNA transcripts.

Another class of models invokes aggregation. At NaCl concentrations near 100 mM, about 20% of a typical chromatin preparation is insoluble (Komaiko & Felsenfeld, 1985); perhaps the differential sedimentation properties we observe could be related to partial (and preferential) aggregation of the inactive chromatin fraction. We have reported earlier (Komaiko & Felsenfeld, 1985) that the aggregation of erythrocyte chromatin fractions in the salt concentration range above 80 mM NaCl involves exchange of histones H1 and H5 and selective precipitation of chromatin possessing the higher molecular weight DNA components regardless of whether they contain globin or ovalbumin gene sequences. We therefore expect that there should be no difference between the aggregation properties of chromatin from active and inactive genes when comparing fractions containing DNA of the same size. Weintraub (1984) has reported that inactive chromatin fractions tend to sediment and electrophorese as aggregates in the salt concentration range from 20 mM upward, but we do not see this behavior in our preparations at 25 or 50 mM NaCl. We therefore think it unlikely that the difference in sedimentation properties we observe can be explained by differential aggregation. It is more reasonable to suppose that the differences arise from a partial depletion of histones H1/H5, core histones, or both.

Although the sedimentation behavior of chromatin in 25 or 50 mM NaCl suggests that active and inactive genes have similar complements of histones, differences of mass of the order of 5–10% might go unnoticed unless they had a large effect on the shape of the particle. Kimura et al. (1983) have suggested that a partial depletion of histones H1 or H5 in their globin chromatin restriction fragments might account for the difference in refolding properties. Although this is not the hypothesis they presently favor (see below), it is an attractive one, since histone H1 is the most readily stripped of the histones, and since partial losses would lead to incomplete folding (Allan et al., 1981). Smith et al. (1984) have presented evidence for a partially disrupted higher order structure in transcriptionally active chromatin and for the possible involvement of histone H1 depletion.

Under the ionic conditions used for these studies, histones H1 and H5 appear to exchange readily among small chromatin fibers (Thomas & Rees, 1983). If transcriptionally active chromatin in our samples is depleted of H1 and H5 despite this free exchange, the chromatin must have an intrinsically reduced affinity for these histones. In an attempt to address this question, Kimura et al. (1983) have measured the effect of addition of excess histone H1 on the sedimentation properties of globin-specific chromatin. They observed no change in the altered sedimentation properties of the β-globin chromatin, suggesting that if this chromatin is depleted of H1 or H5 in vivo, it is because of a reduced binding constant for these histones.

Another possible explanation of our results is that a small number of histone octamers (and their associated H1 or H5 molecules) are missing from the active chromatin. The effect of such a loss on the ability of the 30-nm fiber to refold is not known, but it might well result in the introduction of a defect giving rise to a flexible joint. Such a defect could also serve as a nucleation site for loss of histones H1 and H5, which would tend to dissociate from fiber ends. This would lead to propagation of the disordered state. Alternatively, the remaining nucleosomes could be redistributed evenly along the DNA to produce longer average linker DNA lengths and reduced solenoid stability. Core histones do not exchange under our experimental conditions, and therefore, this model does not require that we postulate a reduced intrinsic affinity of core histones for active chromatin.

Karpov et al. (1984) have observed a progressive loss of histone H1, following by the core histones, associated with increasing levels of transcription in *Drosophila* heat-shock genes that are in the process of being induced. It may be that the globin or ovalbumin gene, transcribed at a relatively low

rate compared to the fully induced heat-shock genes, resembles in structure the heat-shock gene chromatin at early stages of induction.

The β^A-globin gene in chicken erythrocytes has a hypersensitive domain in its 5′-flanking region (McGhee et al., 1981), and the ovalbumin gene in oviduct has several hypersensitive domains (Kaye et al., 1984, 1986). In the case of the β-globin gene, it is known (McGhee et al., 1981) that the domain lacks at least one nucleosome. Furthermore, the β-globin gene has a hypersensitive domain downstream of the coding region, which might also lack a nucleosome (McGhee et al., 1981). Since these are preferred cutting sites for micrococcal nuclease, many β-globin chromatin fragments isolated from erythrocytes will terminate with one or the other, but larger fragments are likely to include at least one, and those generated by *Eco*RI digestion (Kimura et al., 1983) will contain both. Recent results of Caplan, Kimura, Gould, and Allan (personal communication) suggest that, at least in the case of chromatin containing the β-globin gene, loss of nucleosomes from the hypersensitive domains is sufficient to account for the observed change in histone content and hydrodynamic properties. As noted above, the absence of a small number of nucleosomes may be sufficient to destabilize the compact form of the fiber, and in addition promote further loss of histones H1 and H5. It is thus possible that the general partial unfolding of chromatin 30-nm fibers is in some cases caused by the presence of hypersensitive domains.

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