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Differences in Rearrangements of H1 and H5 in Chicken Erythrocyte Chromatin†

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ABSTRACT: H1 can rearrange in chicken erythrocyte polynucleosomes in 80 mM NaCl buffers. These rearrangements have been studied by sedimentation analysis. H1 redistributes between polynucleosomes as well as between polynucleosomes and monosomes. In these rearrangements H1 molecules move to free DNA sites. In contrast to H1, the chicken erythrocyte specific lysine-rich histone H5 does not show any of these dynamic properties. This difference in mobility of H1 and H5 also manifests itself in the selective extraction of H1 from H1, H5 containing polynucleosomes by the cation-exchange resin AG 50W-X2 at 80 mM NaCl.

Histone H1 is involved in the packing of nucleosomes into a 200-300-Å fiber. Different models—a solenoidal architecture (Finch & Klug 1976; Thoma et al. 1979), a higher order unit (superbead) (Hozier et al., 1977), and other packing models (Bradbury, 1977)—have been put forward. From the experimental work leading to various models it has become clear that the observed chromatin superstructure is very dependent on the ionic strength conditions. The recent electron microscopy work of Thoma et al. (1979) shows that in the

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range 0–60 mM NaCl many slightly different well-defined nucleosomal arrangements can be observed provided the chromatin contains H1. These workers were further able to locate H1 at the entry and leaving points of the nucleosomal DNA. In this fashion H1 contributes to the 166 base pair (bp)/particle. Now there exists both a tissue and a species variability in the histone H1 class. This variability in H1 might be important in the process of nucleosomal packing. There exists a very peculiar H1 variant, namely, histone H5. H5 can be considered as a H1 variant due to the extensive amino acid sequence homologies (Isenberg, 1979; Yaguchi et al., 1977, 1979). During erythropoiesis of birds, H5 gradually displaces H1 up to a ratio of about 3:1 in mature erythrocytes (Sung, 1977; Sotirov & Johns, 1972). The H5 accumulation is generally thought to be correlated with the chromatin condensation and transcriptional inactivation observed in mature erythrocyte cells (Andreeva et al., 1978). A characterization of the H1/H5 containing chicken erythrocyte chromatin may contribute to a better understanding of how the H1 class of proteins is involved in the chromatin structuration.

We recently (Muyldermans et al., 1980) described a method allowing removal specifically of H1 from chicken erythrocyte chromatin without detectable loss of H5 or core particle histones and without core particle sliding. This was achieved by treating the chicken erythrocyte chromatin at 80 mM NaCl with the ion-exchange resin AG50W-X2. In this paper we present a characterization of both the native (i.e., untreated) and the resin-treated chromatins. This characterization is done by (a) sedimentation analysis of the obtained chromatins in linear sucrose gradients containing NaCl concentration ranging from 0 to 80 mM NaCl, (b) precipitation analysis of the chromatin in the range 0–600 mM NaCl, and (c) analysis of the behavior of well-defined chromatin fragment mixtures by sucrose gradient centrifugation and by a study of their precipitation behavior in the range 0–600 mM NaCl.

Materials and Methods

Preparation of Fragmented Chicken Erythrocyte Chromatin. Erythrocyte nuclei and fragmented chromatin were prepared as described elsewhere (Muyldermans et al., 1980).

H1 depletion and solubility tests were performed as described by Muyldermans et al. (1980). H1,H5-depleted chromatin was prepared by treatment of chromatin at 600 mM NaCl under identical conditions.

Gel Electrophoresis. Histones were separated on 17.5% acrylamide–NaDodSO$_4$ gels (Wyns et al., 1978).

Preparation of Chicken Erythrocyte Monosomes. Nuclei were prepared in the same way as described for long-fragmented chromatin. In order to produce monosomes the digestion time was extended to 45 min. The digestion was stopped by adding EDTA to a final concentration of 10 mM. The nuclei were pelleted by centrifugation and dialyzed overnight against 10 mM NaCl, 10 mM Tris, 1 mM EDTA, and 10 mM $NaHSO_3$, pH 7.4. After the nuclear debris (3000g, 10 min) was pelleted, the supernatant (6000 units, OD 260 mm) recovered from 100 mL of nuclei (2×10^9/mL) was applied on a 600-mL 60–30% (w/w) sucrose gradient in the Ti-14 Beckman rotor, as described by Wittig & Wittig (1977). The isolated monosomes do not contain any di- or trinucleosomes. Furthermore they show no subnucleosomal degradation.

Preparation of H1- and H1,H5-Containing Monosomes. The early eluting monosome fractions contain, in addition to the core histones, histone H1. These H1-containing monosomes are followed by monosome fractions which show the presence of H1 and H5. (Further details and analysis of this will be published elsewhere.)

Preparation of H5-Containing Monosomes. Micrococcal nuclease fragmented H1-depleted chicken erythrocyte chromatin was dialyzed against 10 mM Tris-HCl, pH 7.4, and 0.1 mM EDTA. After dialysis, the H1-depleted material (OD_{260} = 5) was brought to 0.7 mM $CaCl_2$ and was digested for 15 min at 37 °C by using 10 units/mL micrococcal nuclease (Worthington). The digestion was stopped by adding EDTA up to 5 mM. The digested material was loaded on a linear 10–30% (w/w) sucrose gradient in 10 mM NaCl, 1 mM phosphate, pH 6.8, and 0.2 mM EDTA. In order to exclude contaminating di- and trinucleosomes, a second sucrose gradient centrifugation of this fraction was performed, and the H5-containing monosome fraction is recovered from it.

Preparation of Monosomes Depleted in H1 and H5. When chicken erythrocyte monosomes are resin treated in the same way as described above, both H1 and H5 is taken up by the resin. So the obtained monosomes are free of H1 and H5.

Preparation of Monosomal DNA. This was done by phenol extraction as described by Simpson & Whitlock (1976).

5′-Terminal ^{32}P Labeling of Monosomes. The labeling was done as described by Simpson & Whitlock (1976), the Mg^{2+} concentration was lowered to 1 mM. The [γ-^{32}P]ATP and the polynucleotide kinase were a gift from Dr. Van Montagu.

Preparation of Chicken Erythrocyte Histone H1. A crude H1 and H5 mixture was prepared from chicken erythrocyte nuclei by 5% perchloric acid extraction and acetone precipitation. Histone H1 was then purified on a Bio-Gel P60 column following the manipulation of Böhm et al. (1975).

Sucrose Gradients and Computing Environment. All used sucrose gradients were linear, and they were run in the SW41 Beckman rotor at 5 °C. The type of linear gradient and the run conditions used are indicated in the legends of the figures. Gradients were analyzed by pumping them from the bottom through a turbulence-free flow cell mounted in a Model 101 Hitachi spectrophotometer, whose transmission output is log converted by an Optilab multianalog 201. The Optilab output is then sampled by a 12-bit digital voltage monitor card located in a Hewlett-Packard 6940B multiprogrammer unit. This A/D card is under control of a program operating in a real time executive system. This program stores the sampled data (about 1000/gradient) on disk files. Other programs then access these data files, so it becomes easy to plot the OD profile or to calculate different parameters as, e.g., the weight-average position, or to calculate, in the mixing experiments, theoretical expected profiles.

Results

Comparative Analysis of Sedimentation of Native, H1-Depleted, and H1,H5-Depleted Chromatin as a Function of Ionic Strength. The sedimentation coefficient of polynucleosomes containing H1 increases when the NaCl concentration is raised from 0 to 100 mM (Renz et al., 1979). This increase is accompanied by the induction of higher structuration and compaction of nucleosomal arrays as observed in the electron microscope (Thoma et al., 1979).

We have compared the sedimentation behavior as a function of NaCl concentration in the gradient of (a) H1,H5-containing chicken erythrocyte polynucleosomes or chromatin, (b) H1-depleted H5 containing chromatin, and (c) H1,H5-depleted chromatin. Figure 1A shows the histone proteins of these materials. Figure 1B demonstrates the effectively observed sucrose gradient sedimentation profiles of the three materials obtained from one and the same micrococcal nuclease digest.

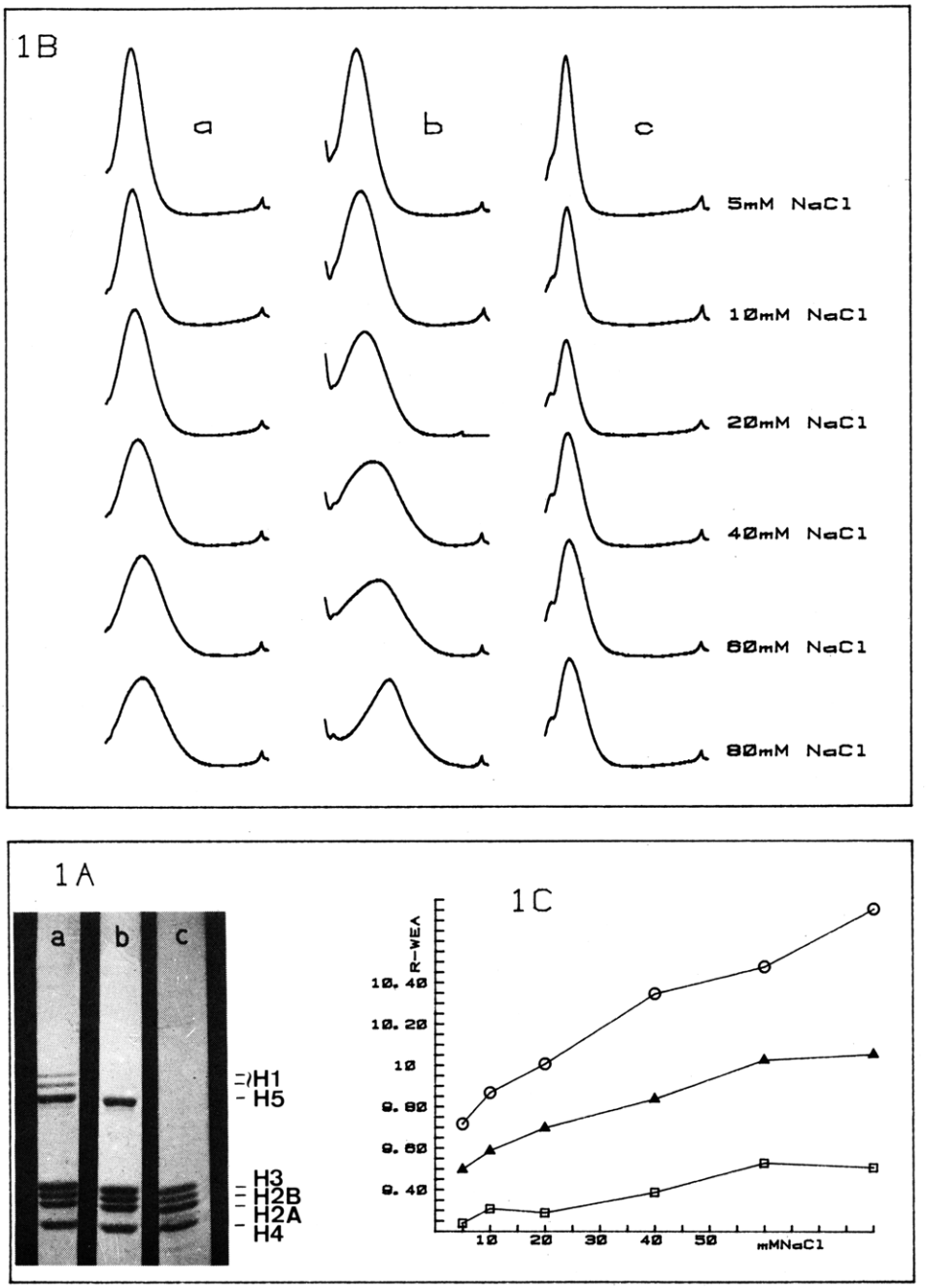

FIGURE 1: (A) Protein NaDodSO$_4$ gel electrophoresis of native chromatin (slot a) and chromatin which was resin treated in 1 mM sodium phosphate, pH 6.8, and 0.2 mM EDTA containing 80 mM NaCl (slot b) or 600 mM NaCl (slot c). The chromatin was resin treated at an OD_{260} = 20 with 0.25 volume of the resin AG50W-X2. (B) Sedimentation profiles in linear sucrose gradients as a function of NaCl concentration in the gradient: (a) H1-depleted fragmented chicken erythrocyte chromatin; (b) native fragmented chicken erythrocyte chromatin; (c) fragmented chicken erythrocyte chromatin depleted in H1 and H5. The profiles are drawn from the top (left) to the bottom (right) of the sucrose gradient. The NaCl concentrations used were 5, 10, 20, 40, 60, and 80 mM NaCl. The sucrose gradient was 9.9 mL and 5–25% (w/w) sucrose. Run conditions: SW41 rotor, 5 °C, 33 000 rpm, 100 min. (C) Evolution of the calculated weight-average position of the profiles drawn in (B): (○) native fragmented chromatin; (▲) H1-depleted fragmented chromatin; (□) fragmented chromatin depleted in H1 and H5. The weight-average position in the SW41 rotor (ordinate) is expressed in centimeters.

Figure 1C expresses these observations numerically. As the polynucleosomes produced by nuclease digestion necessarily are heterogeneous in length, we have plotted the weight-average position in the optical density profiles on the ordinate. Comparing the native with the H1-depleted chromatin, one notes that the difference in weight-average position between these two materials increases with NaCl concentration. The H1-depleted chromatin is quite different from that of the H1,H5-depleted chromatin, the latter being flatter.

The differences observed between the sedimentation behavior of the native and the H1-depleted material suggest that H1 is necessary in the structuration of the chicken erythrocyte chromatin. This prompted us to follow the sedimentation behavior of partially H1-depleted chromatin.

If the ion-exchange resin depletion procedure is carried out below 80 mM NaCl, histone H1 is partially depleted (Muyldermans et al., 1980). The NaDodSO$_4$–protein gel of Figure 2A shows the histone proteins from polynucleosomes, H1 depleted in increasing NaCl and Figure 2B the concomitant change in weight-average position (calculated from the optical density profiles recorded at 260 nm) of these materials. Since the resin-depletion procedure does not affect the repeat length (Muyldermans et al., 1980), these results show that there is a relationship between the amount of H1 in a given

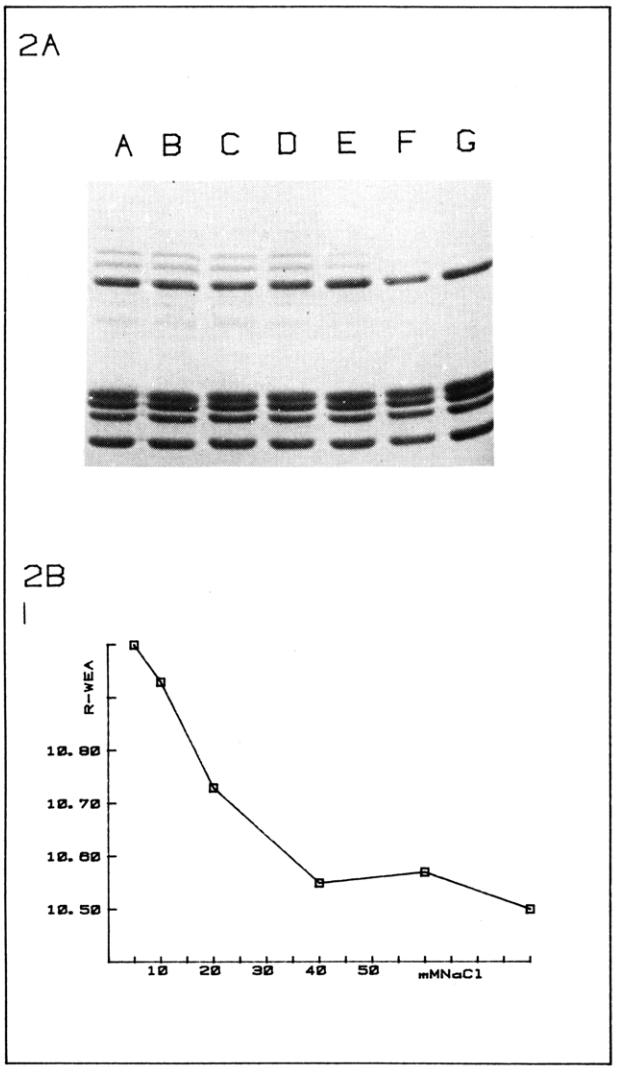

FIGURE 2: (A) Chicken erythrocyte chromatin proteins before (a) and after treatment with the resin in the presence of 5, 10, 20, 40, 60, and 80 mM NaCl (b–g, respectively). (B) Weight-average positions (in cm) in the SW41 rotor of fragmented chicken erythrocyte chromatin as a function of the NaCl concentration used in the AG50W-X2 resin depletion experiment. All gradients were 5–25% (w/w) in 80 mM NaCl, 1 mM sodium phosphate, pH 6.8, and 0.2 mM EDTA. Run condition: SW41 rotor, 5 °C, 38 000 rpm, 90 min.

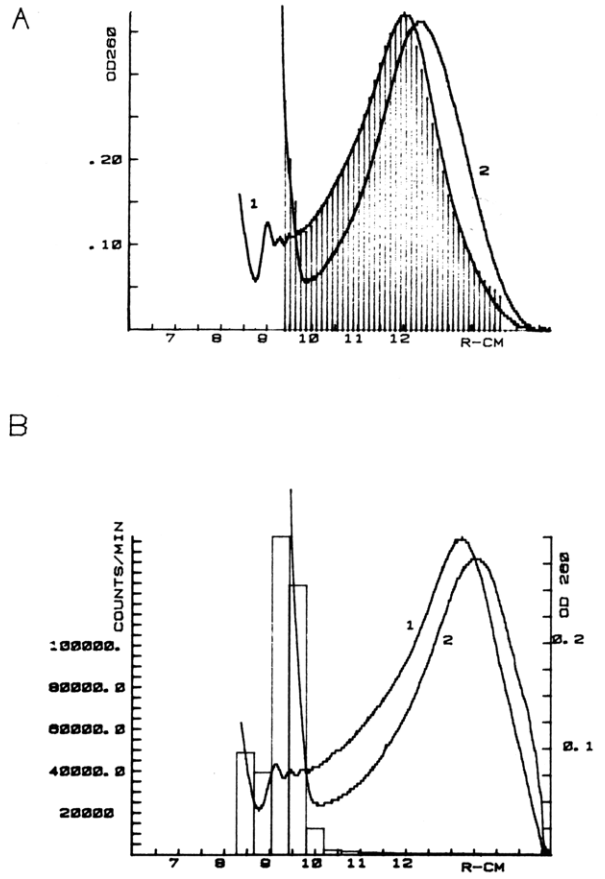

FIGURE 3: (A) Optical density profile of fragmented native chicken erythrocyte chromatin (curve 1) and a mixture (1:1 w/w) of native chromatin and H1,H5-containing monosomes (curve 2). The boundary of thhe shadowed area represents the profile obtained by adding the profiles obtained separately for the native chromatin (curve 1) and the H1,H5-containing monosomes (curve not shown). Sucrose gradients were 10–30% (w/w) in 80 mM NaCl, 1 mM sodium phosphate, pH 6.8, and 0.1 mM EDTA. Run condition: SW41 rotor, 38 000 rpm, 5 °C, 165 min. (B) Radioactivity and optical density (OD) profiles of a mixing (1:1 w/w) experiment of native fragmented chicken erythrocyte chromatin and 5′-^{32}P-labeled H1,H5-containing monosomes: (curve 1) OD profile of native fragmented chromatin; (curve 2) OD profile of the mixture; (histogram) radioactivity profile of curve 2. The OD profiles refer to the right ordinate; the radioactivity profile is the left ordinate. We used a 10–30% (w/w) sucrose gradient in 80 mM NaCl, 1 mM sodium phosphate, pH 6.8, and 0.2 mM EDTA. Run condition: SW41 rotor, 38 000 rpm, 5 °C, 165 min.

chicken erythrocyte chromatin sample and its sedimentation behavior. The question may be raised whether or not in a partially depleted chromatin H1 molecules redistribute in the 80 mM NaCl chromatin. This reasoning brought us to a second kind of experiment, namely, the analysis of well-defined chromatin fragment mixtures.

Analysis of Mixtures of Chromatin and H1,H5-Containing Monosomes. Chicken erythrocyte chromatin and chicken erythrocyte H1,H5-containing monosomes were mixted at 4 °C in 80 mM NaCl, 1 mM phosphate, pH 6.8, and 0.2 mM EDTA at a 1:1 weight ratio. After an incubation period of 15 min they were applied on linear sucrose gradients in the same ionic conditions. Figure 3A shows the recorded optical density profiles of the native isolated chromatin (curve 1) and the mixing experiment (curve 2). It is clear that a shift occurs toward the bottom of the tube. Before planning other mixing experiments, one should point out that the observed shift cannot be accounted for by a simple addition of the profiles obtained separately for the native material (curve 1) and for thee added monosome (curve not shown). When these data files are added, no chromatin peak shift is seen (shadowed curve), so the observed shift is significant. It is possible to give different explanations. On one hand, there might be aggregation of monosomes to our chromatin material [in the case of oligonucleosomes, this was put forward by Strätling (1979)]; on the other hand, there could be a displacement of histones H1 and/or H5 from the added monosomes to the higher material.

In order to test the first hypothesis, we radiolabeled the 5′ termini of the H1,H5-containing monosomes using the [γ-^{32}P]ATP–polynucleotide kinase technique (Simpson & Whitlock, 1976). After the labeled monosomes and the native chromatin were mixed in 80 mM NaCl, a sucrose gradient centrifugation in 80 mM NaCl was performed. The optical density profile and radioactivity were monitored in parallel. Figure 3B shows the superimposed optical density and radioactivity profiles obtained. Since the OD profile again shows a downward shift of the chromatin peak, we may conclude that this shift is not destroyed by labeling monosomes. The radioactivity profile proves that in our experimental conditions there is no aggregation of monosomes with our chromatin material. Indeed all the labeling is found in the monosome

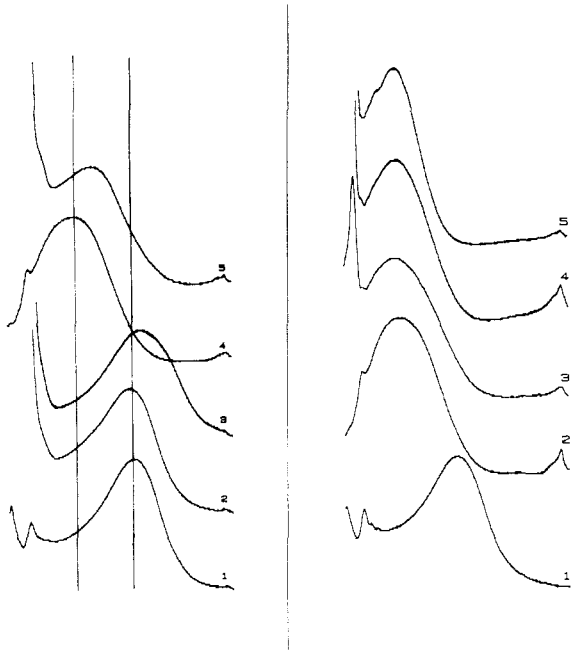

FIGURE 4: (A, left) Optical density profiles of native fragmented chicken erythrocyte chromatin (curve 1) mixed at a 1:1 w/w ratio in 80 mM NaCl, 1 mM sodium phosphate, pH 6.8, and 0.2 mM EDTA with H5-containing monosomes (curve 2), H1-containing monosomes (curve 3), and monosomes depleted in H1 and H5 (curve 5). Curve 4 is the profile for the H1-depleted fragmented chromatin. The profiles are drawn from the meniscus (left) to the bottom (right) of the gradient (10–80% sucrose in the same buffer as used in the mixing experiment). The vertical lines represent the peak positions of the H1-depleted chromatin (left) and the native chromatin (right). Run conditions: SW41 rotor, 38 000 rpm, 5 °C, 165 min. (B, right) Optical density profiles of the native fragmented chicken erythrocyte chromatin (curve 1) mixed with monosomal DNA at the DNA/weight ratio 0.1 (curve 1), 0.4 (curve 4), and 1 (curve 5). Curve 2 represents the obtained profile for the H1-depleted chromatin. The profiles are drawn from the meniscus (left) to the bottom (right) of the sucrose gradient (10–30% in 80 mM NaCl, 1 mM sodium phosphate, pH 6.8, and 0.2 mM EDTA). Run conditions: SW41 rotor, 38 000 rpm, 5 °C, 135 min.

region of the gradient. Some component of the added monosomes has moved to the longer chromatin, thereby inducing a sedimentation increase. It is most likely that the H1 and/or H5 may be involved in this displacement. To test this hypothesis, we used in the addition experiments, described further, chicken erythrocyte monosomes containing either H1 or H5. If different results are observed when those two types of monosomes are used, one might suggest which histone (H1 or H5) produces the observed effect.

Addition of H1-Containing Monosomes and of H5-Containing Monosomes. Mixings and gradient analysis were performed as described above. In Figure 4A, curves 1–3 are the OD tracings of respectively native chromatin, the mixture of this chromatin with the H5-containing monosomes, and the mixture with H1-containing monosomes. It is clear that if the monosomes used in the addition experiment lack H1 (but contain H5) no shift is observed, whereas H1-containing monosomes do produce an analogous shift as observed in the mixing experiment with H1,H5-containing monosomes. This suggests that H1 is able to move to the 80 mM NaCl higher material, thereby altering the sedimentation behavior of the chromatin. Of course these results do not exclude the possibility that H5 rearranges also to the longer material, but they do exclude that in such a case H5 alters the sedimentation behavior of the chromatin.

It has been proposed in the case of bovine lymphocyte chromatin (Renz et al., 1977) that in the NaCl concentration

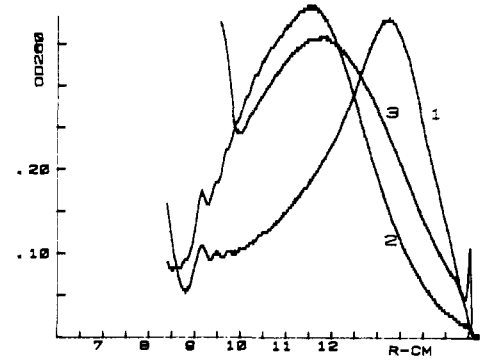

FIGURE 5: Optical density profile of native (curve 1) and H1-depleted (curve 2) fragmented chicken erythrocyte chromatin and a 1:1 w/w mixture of H1-depleted chromatin and H1,H5-containing monosomes (curve 3). Sucrose gradients (10–30%) were in 80 mM NaCl, 1 mM sodium phosphate, pH 6.8, and 0.2 mM EDTA. Run conditions: SW41 rotor, 5 °C, 38 000 rpm, 165 min.

we use, histone H1 might redistribute in a cooperative fashion to larger chromatin. This might explain our addition experiments. In order to see whether this preference of H1 for larger chromatin remains valid in the case of chicken erythrocyte chromatin, we decided to perform another kind of addition experiment, namely, the addition to native material of short DNA and of monosomes depleted in H1 and H5.

Addition of Short DNA and of H1,H5-Depleted Monosomes. Again the mixing was performed in 80 mM NaCl, 1 mM phosphate, pH 6.8, and 0.2 mM EDTA at 0.1 g, 0.4 g, and 1 g of monosomal DNA/g of chromatin DNA. Figure 4B shows the recorded OD profiles obtained after a sucrose gradient centrifugation in 80 mM NaCl. From the tracings it is clear that even at the 0.1 DNA weight ratio the chromatin peak shifts to the position of the H1-depleted chromatin (curve 2). It is interesting to know whether an analogous effect might be produced in an addition experiment using monosomes depleted in H1 and H5 which are likely to have some free DNA sites.

As described under Materials and Methods, a resin treatment of H1,H5-containing monosomes under the same experimental conditions used for the selective H1 depletion of the higher chromatin results in a complete depletion of H1 and H5. Mixing of these monosomes with longer chromatin at a 1:1 weight ratio results in a profile presented in Figure 4B, curve 5. A dramatic shift occurs toward the peak of H1-depleted chromatin (curve 4, Figure 4A). These results show that in the chicken erythrocyte chromatin some component of the higher chromatin migrates toward free DNA sites of the shorter material. In view of the experiments described so far, and in particular the resin treatment experiment of longer chromatin fragments, histone H1 seems to be the most likely candidate.

We now ask if we can regenerate the sedimentation profile of the native chromatin by adding free purified H1 or H1,H5-containing monosomes to the H1-depleted chromatin?

Addition of Purified H1 and of H1,H5-Containing Monosomes to H1-Depleted Chromatin. Figure 5, curve 3, represents the optical density of a 1:1 weight mixture in 80 mM NaCl of H1-depleted chromatin and H1,H5-containing monosomes. One observes only a slight shift from the H1-depleted chromatin (curve 2) toward the peak of the native chromatin (curve 1). This result is not too surprising since we noticed earlier that components of the longer chromatin may move to the shorter material, so there may be an equilibrium distribution of the H1 and/or H5 molecules between the monosomes and the higher material. This may inhibit a

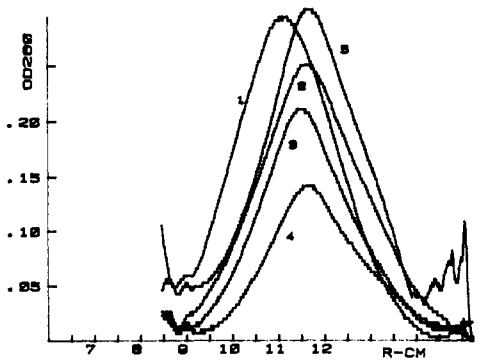

FIGURE 6: Optical density profiles of 1.6 OD_{260} units of H1-depleted chromatin (curve 1) mixed in 80 mM NaCl with 15 μg of H1 (curve 2), 17.5 μg of H1 (curve 3), and 22.5 μg of H1 (curve 4). Curve 5 is the profile of the native chromatin. Sucrose gradients (10–30%) were run in the SW41 rotor for 90 min at 5 °C, 38 000 rpm.

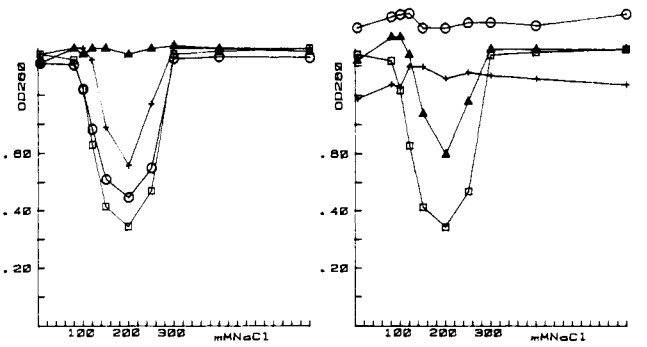

FIGURE 7: (A, left) Precipitation analysis of (□) native chicken erythrocyte chromatin, (▲) mixture of 50% (w/w) native chromatin and H1-depleted chromatin, and (+) mixture of 20% H1-depleted chromatin and 80% native chromatin. (○) Theoretical expected curve in the mixture of 20% H1-depleted and 80% native chromatin. The ordinate represents the OD of the supernatant obtained after centrifugation of these mixtures, which were kept at 4 °C for 5 h. (B, right) Precipitation analysis of (□) native chicken erythrocyte chromatin, (▲) mixture of 5% monosomal DNA and 95% nature chromatin, (○) mixture of 20% monosomal DNA and 80% native chromatin, and (+) H1-depleted chromatin. The mixtures were kept at 4 °C for 3 h and subsequently centrifugated. The ordinate represents the OD of the obtained supernatants.

massive transfer of H1 and/or H5 to the higher chromatin material. In view of this reasoning, we decided to mix purified H1 with H1-depleted chromatin.

The mixtures used all contained 1.6 OD_{260} units of chromatin but varied in H1 amount—15, 17.5, and 22.5 μg of H1. After an incubation period of 15 min, the mixtures were applied to sucrose gradients in 80 mM NaCl. The resulting monitored profiles are shown in Figure 6. One notices that the reconstituted peak is very near the native one, but as the amount of added H1 is increased beyond 15 μg of H1 there is a rapid decrease in the amount of chromatin material which can be recovered from the gradient. In contrast to the addition experiment of H1,H5-containing monosomes with native chromatin, this reconstitution experiment never produces a chromatin peak running faster than the native one: the weight-average positions for the native fragmented chromatin and the chromatin reconstituted with 22 μg of H1 (Figure 6) are respectively 11.81 and 11.80. If some component, most likely H1, can move from higher chromatin material, we may ask whether this is also the case in a mixture containing only larger chromatin fragment lengths. Is the sucrose profile of an H1-depleted chromatin mixed with native chromatin generated by simple addition of the profiles obtained in the individual chromatin components? We tried to answer this question by using this approach, but such a mixture of course results in the appearance of a single rather broad peak in the sucrose gradient which makes it difficult to observe accurately deviations from theoretically expected curves. However, there is another technique which makes it possible to analyze this kind of mixture. As we described elsewhere (Muyldermans et al., 1980), the H1,H5-containing chicken erythrocyte chromatin has a minimal solubility at 200 mM NaCl whereas the H1-depleted chromatin is perfectly soluble over the whole range from 0 to 600 mM NaCl. Analyzing the proposed kind of mixture in a precipitation analysis may therefore reveal the required answer.

Precipitation Analysis. Precipitation analysis was carried out as described under Materials and Methods. As before, prior to the precipitation analysis the mixture was made by adding together in 80 mM NaCl the components involved. The following mixtures were analyzed (the given percentages are percentages expressed in DNA weight of the indicated component in the final mixture): (a) 5% and 20% monosomal DNA with respectively 95% and 80% native chromatin; (b) 20% and 50% H1-depleted chromatin with respectively 80% and 50% native chromatin.

At an input of 20% monosomal DNA or 50% H1-depleted chromatin the mixture becomes completely soluble, as shown in Figure 7A,B. When those mixtures are produced, the concentration of the native chromatin decreases, since we always assay the same amount of material (approximatively 1 OD_{260} unit). Thus one may argue that if the precipitation curve is concentration dependent, the observed data lack any significance. However we proved (data not shown) that there is no change in the precipitation curve in the dilution range we work in. The data obtained for the 20% monosomal DNA and the 50% H1-depleted chromatin are significant. Since the solubility of the chromatin is correlated with the presence of H1 in the material (H1 depletion results in complete solubility; see Figure 7B), we suggest that histone H1 can move to free sites which may be found on monosomal DNA or, more important, H1-depleted chromatin. If the input percentages are lowered, we get an intermediate result. A precipitation curve is observed (Figure 7A,B) that has a minimum at 200 mM NaCl, but the amount of soluble material has increased. This is quite obvious in the 5% monosomal DNA input. One might object to an analogous conclusion for the 20% H1-depleted chromatin input since at all points 20% of the material may be soluble, thereby provoking a nonnegligible upward shift of the curve. We therefore calculated (see Figure 7A) the expected precipitation curve assuming the precipitation curves for both types of chromatin components are additive. From Figure 7A it is seen that the experimentally observed solubility cannot be explained on a basis of simple additivity. It is important to note that the input of 20% monosomal DNA leads to a complete solubility, whereas an intermediate solubility is observed when an input of 20% H1-depleted chromatin is used. This might be correlated with the obvious fact that in the H1-depleted chromatin the amount of possible free sites is much lower than in free DNA.

Discussion

The selective H1 depletion from H1,H5-containing polynucleosomes, described and discussed in an earlier paper (Muyldermans et al., 1980) was presented as a consequence of an intrinsic higher affinity of H5 for DNA and a more dynamic behavior of H1 in the ionic strength range of our studies. This we tried to correlate with the higher basicity and the relatively higher arginine/lysine ratio of H5, and very recently other workers have shown that indeed *all* the H5

domains (N-terminal, central, and C-terminal regions) bind more strongly to DNA than the corresponding H1 domains (Aviles et al., 1979).

We think the data derived from the analysis of polynucleosome–monosome mixtures described in this paper may add further weight to the direct and simple hypothesis that H1 has a mobile character in chicken erythrocyte chromatin: addition of H1-containing monosomes to the native chromatin produces a downward shift (sedimentation increase; see Figure 4A); addition of H5-containing monosomes does not produce any chromatin peak shift (see Figure 4A). Thus we propose, although we cannot exclude some effects being due to nonhistone proteins, that H1 moves from the shorter to the longer material, and vice versa: H1 may move to short free monosomal DNA and hypothetical free DNA sites of H1,H5-depleted monosomes; the precipitation analysis of the mixture containing 50% H1-depleted and 50% native chromatin suggests that H1 can also move from native longer material to free sites in longer material.

We feel the reader may wonder why we apparently neglected to fractionate the sucrose gradient obtained after running the various mixtures described. Indeed such a fractionation may lead to a direct proof that H1 is involved in a displacement reaction. Unfortunately this does not work out because the different peaks in the optical density profiles are not sufficiently resolved for quantative analysis. Furthermore, the proposed H1 displacement most likely involves a fractional rather than a total H1 displacement. Although the simple proposal of a more mobile H1 and an intrinsically more "sticky" H5 may explain most of the results, we think some of the data require a more subtle interpretation and perhaps additional concepts.

The native chromatin peak position is not reconstituted by adding H1,H5-containing monosomes to the H1-depleted chromatin in a 1:1 w/w ratio whereas addition of purified H1 can reconstitute largely (but not completely) the native chromatin peak position. It is important to note (Figure 6) that after further addition of H1 there is no further chromatin peak shift but a gradual chromatin precipitation. Only the addition of monosomes containing H1 and H5 or monosomes containing H1 to native chromatin produces a sedimentation increase of the native material. These observations may be rationalized as follows. Nehlson et al. (1979) recently showed that in the case of bovine thymus chromatin the polynucleosomes possess a second H1 binding site per histone octamer. This site is not occupied in the native chromatin, and it was indicated that H1 binding to this site is more efficient in polynucleosomes than in mono- and dinucleosomes. Therefore if one adds purified H1 to an H1-depleted chromatin, one might expect that beyond a certain amount of added H1, H1 may bind cooperatively without marked length preference to the polynucleosome population so that part of the chromatin precipitates, while the peak position of the remaining chromatin is unaltered. In such a reasoning there may be a peak shift in the mixture of native chromatin with H1,H5-containing monosomes. Indeed, in this case the addition of H1 is accompanied by a proportional DNA addition (part of the DNA of the added monosomes). Since in the mixture this DNA itself may compete for H1 binding, it may weaken the extent of cooperativity, meaning that although there is still a net H1 displacement toward the longer material, this extra H1 is distributed in a more or less homogeneous way, thus inducing a sedimentation increase of the chromatin.

So far we did not discuss the H1,H5 depletion of chicken erythrocyte monosomes. Although this depletion may be due to possible free histone tails in monosomes (Cary et al., 1978), we do feel that in the light of this observation part of the above stated conclusions require further comments.

All our results were discussed on a basis of intrinsic DNA-binding differences of H1 and H5. It has been suggested, however, that in the 250-Å fiber the H1–H1 interactions can play a fundamental role (Thoma et al., 1979). It is possible that in the 80 mM NaCl chicken erythrocyte chromatin H1–H5 interactions, for example, may be much weaker than H1–H1 or H5–H5 interactions. If in 80 mM NaCl histones H1 and H5 are organized as interspersed lattices along the chromatin fiber, the mean H1 lattice length may be smaller than a critical size to resist H1 depletion. On the basis of the observed H1:H5 ratio one might expect H5 lattices to be more extended. The observation that both H1 and H5 are depleted in monosomes might be interpreted on these grounds.

In view of this reasoning, H1 may acquire a dynamic character in the chicken erythrocyte chromatin. Thus, the proposed difference in behavior between H1 and H5 may be due to an intrinsic difference in DNA binding affinity and/or an effect of the H1,H5 distribution along the chromatin fiber.

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