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Biochemistry

Involvement of histone H1 in the organization of the chromosome fiber

(structural transition/ionic strength effects/cooperative binding/nucleosomes)

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ABSTRACT **At high ionic strength (e.g., physiological salt concentrations) chromosome fibers are 200 Å in diameter and composed of discrete globular structures that are held together by histone H1. At low ionic strength the fibers unfold and appear as the familiar chains of nucleosomes (80 Å in diameter). The unfolding of chromosome fibers occurs within a narrow salt range. It results from a change in the mode of the interaction between histone H1 and the chromosome fiber and is very likely the consequence of a change from cooperative binding between histone H1 and DNA to a noncooperative binding. In the noncooperative binding state histone H1 molecules are randomly redistributed along the chromosome fiber.**

Chromosomes consist of fibers which are about 200–250 Å thick (1–3). These fibers unfold at low ionic strength into thinner fibers (1, 4–6) with a diameter of 80–100 Å. The basic structural unit (the nucleosome) of the thin fiber is a complex of histones H2A, H2B, H3, and H4 with about 200 base pairs of DNA (7, 8). Histone H1 does not contribute to the structure of the nucleosome (9). Because both chromosomes and DNA–histone H1 complexes decondense at low ionic strength (10, 11), histone H1 may be involved in the coiling or folding of nucleosome chains to form the 200- to 250-Å fiber. Experimental evidence for such an involvement of histone H1 is given in this report. It is shown in these and related studies* that the 200- to 250-Å "knobby" fiber seems to be made of groups of several nucleosomes held together in discrete globular structures by histone H1.

This study was in part prompted by earlier studies (12) which showed that the mode of histone H1 binding to DNA undergoes a salt-dependent transition (near 20 mM NaCl or 0.8 mM $MgCl_2$). Below this salt concentration histone H1 is randomly bound to DNA molecules irrespective of their size and base composition and DNA–histone H1 complexes retain a relaxed form similar to that of DNA without protein. Above that transition salt concentration and in the presence of excess DNA, histone H1 binds to some DNA molecules and not to others (cooperative binding). The histone-H1-bound DNA exhibits a more compact (folded) form. Cooperative binding has been shown to occur preferentially on large and (A+T)-rich DNA molecules. It will be shown in this report that histone H1 on chains of nucleosomes exhibits binding behavior similar to that on naked DNA, and that it is the cooperative binding of histone H1 that is responsible for the folding of the thin chromosome fiber. The results of four independent experimental approaches provide evidence that supports this view: (*i*) competition between long and short nucleosome chains for histone H1 analyzed by filter binding; (*ii*) the distribution of histone H1 in mixtures of long and short chromosome fibers separated by sucrose gradient velocity sedimentation; (*iii*) the sedimentation behavior of long chromosome fiber fragments as a function of NaCl concentration in the range of the transition; (*iv*) electron microscopy of chromosome fibers above and below the transition.

* J. Hozier, M. Renz, and P. Nehls, unpublished data.

MATERIALS AND METHODS

Cell nuclei were isolated from bovine lymphocytes (13) and stored in 2 mM $MgCl_2$, 5 mM Tris·HCl (pH 7.5), and 66% (vol/vol) glycerol at −60° until needed, but not longer than 10 days. Radioactively labeled nuclei were obtained from bovine lymphocytes that had been stimulated by phytohemagglutinin P (Difco) in medium containing [^{3}H]thymidine after 60 hr of incubation.

Fragmentation of chromatin in nuclei by micrococcal nuclease (Boehringer or Worthington) was carried out at 0° in 0.2 M sucrose, 1 mM $CaCl_2$, 5 mM Tris·HCl (pH 7.5), and either 80 or 60 mM NaCl at a concentration of $2.4 \cdot 10^8$ nuclei per ml. The digestion reaction was terminated and nuclei were lysed by gently adding the same volume of a solution containing 5 mM EDTA, 5 mM Tris·HCl (pH 7.5), and 80 or 60 mM NaCl. The nuclear debris was pelleted at 5000 × *g* for 8 min. The supernatant contained 50–80% of the nuclear DNA in the form of fragmented chromosome fibers. Histone-H1-depleted fiber fragments were made by the aid of tRNA (14). Histone H1 was prepared by the trichloroacetic acid extraction procedure (15).

The filter binding assay was carried out as described earlier (12). Stock solutions of histone H1 were diluted into 0.5 ml samples of 5 mM Tris·HCl (pH 7.5) and 40 mM NaCl containing equal weights of labeled and unlabeled histone-H1-depleted nucleosomes. After incubation for 30 min at 0°, the reaction mixture was filtered through nitrocellulose membrane filters at a flow rate of 0.1 ml·sec^{-1}. The filters were washed three times with 0.7 ml of buffer, dried, and monitored for radioactivity. The values given are the means of three experiments; the standard deviations were less than 10% of the mean values. Filters retained 20 (±5)% of the histone-H1-depleted nucleosome trimer (background).

Sucrose gradient analyses were made by layering fragmented chromosome fiber samples (0.5 ml) on preformed linear gradients from 10 to 30% sucrose containing 1 mM sodium phosphate (pH 6.8), 0.2 mM EDTA, and NaCl at the same concentration as in the samples. Nitrocellulose tubes with 0.5 ml cushions of 86% (vol/vol) glycerol were spun in a Beckman SW 40 rotor at 3°. The gradients were analyzed with the use of a turbulence-free flow cell (ISCO).

DNA sizes were analyzed electrophoretically on 1.4% agarose gels as previously described (16). In all cases fragments of phage fd replicative form (RF) DNA (17) (kindly supplied by H. Schaller), prepared by digestion with *Hpa*II restriction endonuclease, were run in parallel as size markers.

Proteins were analyzed by sodium dodecyl sulfate/polyacrylamide gel electrophoresis according to Laemmli (18),

except that we used 5 and 12% acrylamide for the stacking and separation gel, respectively.

For electron microscopy, chromosome fibers were fixed with 0.2% (vol/vol) glutaraldehyde in the presence of 10 mM or 70 mM NaCl buffered with 0.5 mM triethanolamine at pH 8.0 and prepared by negative staining with uranyl acetate. Details of the preparations will be given elsewhere* †.

RESULTS

Histone H1 Binding Preference. In analogy to experiments with naked DNA (12) we investigated the size dependence of the interaction between histone H1 and nucleosome chains of various lengths. Competition experiments were performed with a filter binding assay (19). This assay is possible because we find that most short chains of nucleosomes depleted of histone H1 pass through nitrocellulose filters, whereas their complexes with H1 are retained (see *Materials and Methods*). Equal masses of radioactively labeled histone-H1-depleted nucleosomes (chain length = 3) and unlabeled histone-H1-depleted nucleosome chains of various lengths were allowed to compete for histone H1 and the mixture was subsequently filtered (Fig. 1). There is a gradual decrease of radioactivity on the filters when unlabeled nucleosome chains of increasing length are used until a plateau is reached at a chain length of about 7. This indicates that hepta- or octanucleosomes compete more effectively for histone H1 than tri- or dinucleosomes. The binding of histone H1 to longer chains of nucleosomes is therefore more stable than to chains containing fewer nucleosomes.

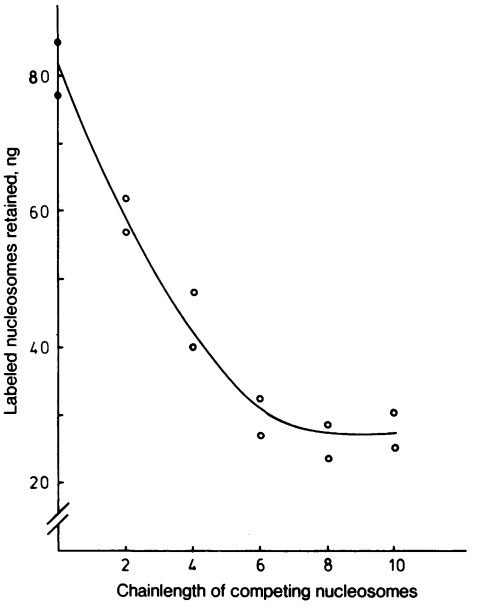

FIG. 1. Nitrocellulose filter assay of the competition between small and large histone-H1-depleted nucleosome chains for histone H1. Histone H1 (40 ng) was added to 0.5 ml of 40 mM NaCl, 5 mM Tris·HCl (pH 7.5), 0.2 mM EDTA containing 150 ng of H1-depleted ^{3}H-labeled nucleosomes (chain length 3) and 150 ng of unlabeled H1-depleted nucleosome chains of various lengths.

Distribution of Histone H1 on Oligonucleosomes. The experiments above suggest that upon fragmentation of chromosomes histone H1 might be redistributed among the fragments such that small fragments lose their histone H1 whereas H1 is enriched on larger fragments. To test this possibility, interphase chromosomes in lymphocyte nuclei were fragmented with the aid of micrococcal nuclease. These fragments are soluble in 80 mM NaCl† in which chromatin prepared by standard techniques at low ionic strength (often water) forms a precipitate. The distribution of histone H1 on fiber fragments of different lengths was examined by sucrose gradient centrifugation and fractions from the gradient were analyzed for histones and DNA size (Fig. 2*A*). The fragments that sediment more slowly represent nucleosomes of shorter chain length and they have the full complement of histones H2A, H2B, H3, and H4, but little or no histone H1. There is a progressive increase in the amount of histone H1 with increasing nucleosome chain length up to about 5 to 6 (Fig. 3). Larger, faster sedimenting fragments contain all five histone fractions in their expected ratio. We conclude that histone H1 is nonrandomly distributed on fragmented chromosome fibers if some of them are shorter than a hexanucleosome, with little H1 bound to small fragments.

If there is a correlation between the unfolding of chromosomes that occurs at low ionic strength (10) and the change in the mode of histone H1 binding to naked DNA near 20 mM NaCl (12), the longer chromosome fiber fragments presumably folded by histone H1 should unfold at low ionic strength and consequently sediment more slowly, while fragments depleted of histone H1 would change their sedimentation behavior only insignificantly in the same ionic strength range (see below). To test these possibilities, an aliquot of fragmented chromosome fibers was dialyzed to low ionic strength (5 mM NaCl) and analyzed again by sucrose gradient centrifugation (Fig. 2*B*). The sedimentation profile is distinctly different from that obtained at 80 mM NaCl. The characteristic pattern of nucleosomes of shorter chain length is much less pronounced due to a drastic decrease in sedimentation velocity of the originally faster sedimenting fragments at higher ionic strength. Analysis of the gradient fractions for histones and DNA size (Figs. 2*B* and 3) reveals that all fragments (with the possible exception of mononucleosomes) contain similar amounts of all five histones, irrespective of their size. This shows that the size preferential interaction of histone H1 vanishes at low ionic strength and that histone H1 is randomized over the whole population of fragments.

Sedimentation Behavior of Large Chromosome Fiber Fragments. Fig. 4 shows the effect of NaCl concentration on the sedimentation rate of large fragments (average length of about 30 nucleosomes) that were obtained by mild nuclease digestion. The average sedimentation coefficient of fiber fragments decreases insignificantly between 60 and 35 mM NaCl. However, as the NaCl concentration was further decreased from 35 to 10 mM NaCl the sedimentation coefficient dropped dramatically from 88 S to 54 S. Such a sharp change in sedimentation behavior would be expected if it reflected a histone-H1-mediated folding–unfolding transition. In agreement with this proposal is the observation that only a small change in sedimentation coefficient could be observed between 40 and 10 mM NaCl when histone-H1-depleted fiber fragments were used (Fig. 4). Moreover, reconstituted chromosome fibers made from H1-depleted fragments and exogeneous H1 showed a similar characteristic sedimentation behavior as native fiber fragments (Fig. 4). We conclude that the sharp decrease in sedimentation velocity between 35 and 10 mM NaCl is caused by an unfolding of chromosome fiber fragments resulting from a loss of histone H1's ability to act as a packing protein.

Electron Microscopy of Large Chromosome Fiber Fragments. To establish a structural correlation between the folded and unfolded state of chromosome fibers, electron microscopic studies were performed which will be described in detail elsewhere* †. Electron micrographs of large fragments at 70 mM

† P. Nehls, J. Hozier, and M. Renz, unpublished data.

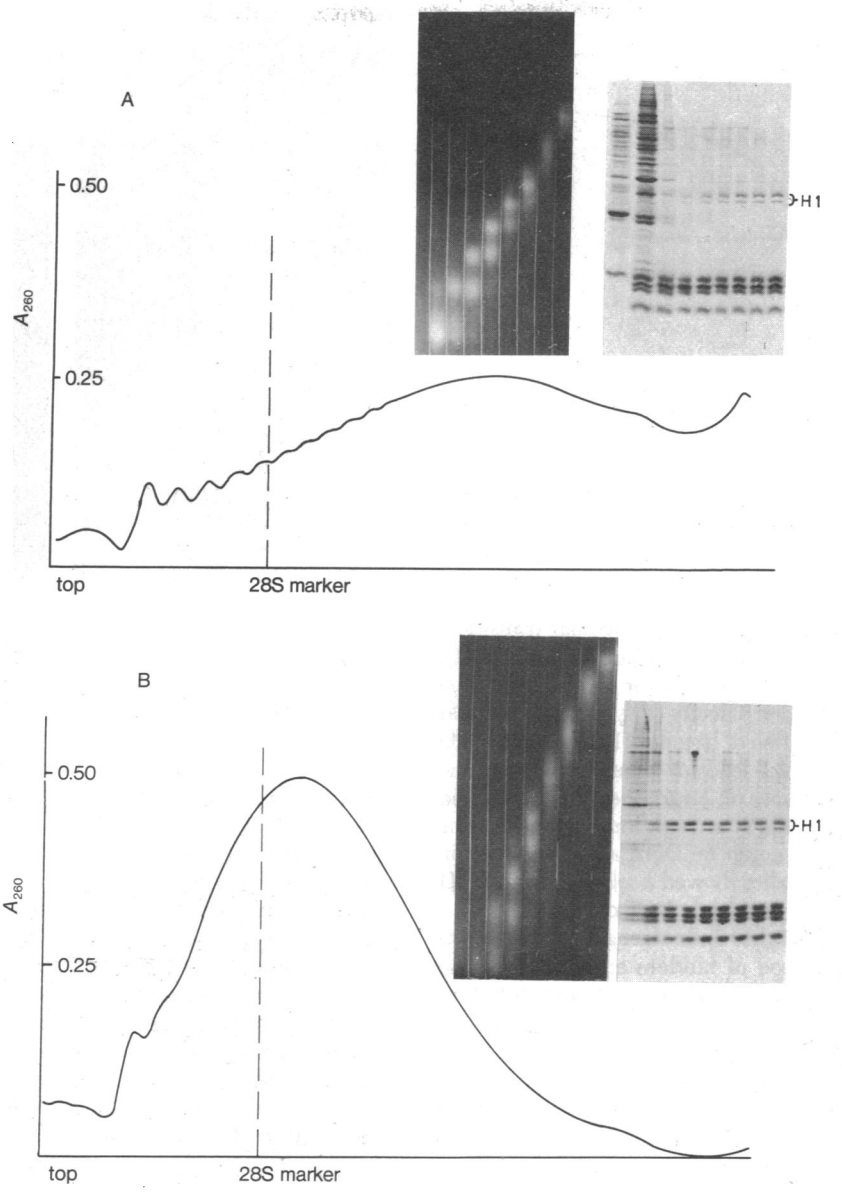

FIG. 2. Sucrose gradient analysis of fragmented chromatin fibers (average nucleosome chain length = 10) at 80 and 5 mM NaCl. Lymphocyte nuclei were digested with 2 units of micrococcal nuclease per $1 \cdot 10^6$ nuclei for 20 min at 0°. After lysis, the supernatant of a low-speed centrifugation (80% of total chromatin) was divided into two portions which were dialyzed against 1 mM sodium phosphate (pH 6.8), 0.2 mM EDTA, and either 80 mM NaCl or 5 mM NaCl. Aliquots (6 A_{260} units; 1 A_{260} unit is the amount of material giving an A_{260} of 1 when dissolved in 1 ml and the path length is 1 cm) were sedimented through sucrose gradients in the same buffers as the samples at 39,000 rpm for 4.5 hr. Those fractions having sedimentation coefficients of about 50 S and less were analyzed electrophoretically for DNA size (*left insets*) and protein content (*right insets*). The left-most gel samples in the insets are from the top of the gradients and the right-most, from the 50S region. (*A*) 80 mM NaCl; (*B*) 5 mM NaCl.

and 10 mM NaCl are shown in Fig. 5. At 70 mM NaCl they appear as "knobby" fibers about 200 Å in diameter (Fig. 5*A*). In contrast, at 10 mM NaCl fragments are, as expected, unfolded and appear as nucleosomes with a diameter of about 80 Å (Fig. 5*B*). Thus, a structural change in the chromosome fiber can be seen in the electron microscope which corresponds well with the biochemical data.

In high salt there is a visible repeat along the fiber axis with a globular appearance. Frequently it can be seen that these 200 Å globular structures are composed of substructures of about 80 Å in diameter, the size of nucleosomes (4, 5). Four or five nucleosomes per knob are visible; due to the globular appearance of the knobs this is the lowest number and 6 to 10 nucleosomes per knob is a more reasonable estimate. An estimate of 6 to 10 is consistent with the lack of size dependence of the nucleosome–histone H1 interaction on oligonucleosomes with a chain length between 6 and 9 (Fig. 1).

DISCUSSION

In these studies the cooperative nature of the nucleosome–histone H1 interaction is most convincingly demonstrated by the preferential binding of histone H1 to nucleosome chains of greater length (Figs. 1 and 2*A*). The preference for larger sizes vanishes at low ionic strength and the binding of histone H1 is random with respect to nucleosome chain length (Figs. 2*B* and 3). Moreover, one can learn from these experiments that in future studies on fragmented chromosome fibers the redistribution of histone H1 has to be taken into account when their fragment length is shorter than 6 to 7 nucleosomes. On large

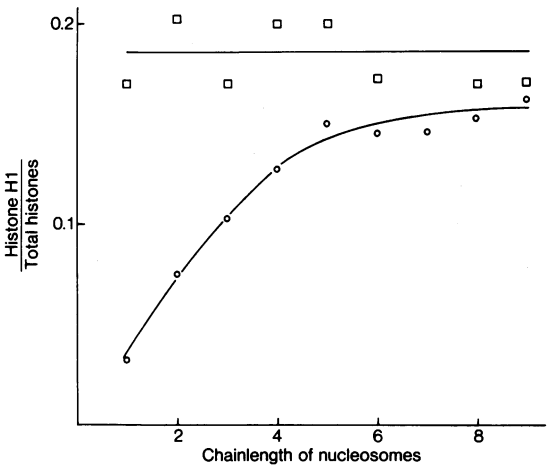

FIG. 3. Distribution of histone H1 on oligonucleosomes in buffers of lower and higher ionic strength. The plots were constructed from densitometer traces of the protein gels shown in Fig. 2. (□) 5 mM NaCl and (○) 80 mM NaCl.

fragments, of course, the presumed intramolecular transition from cooperative to noncooperative binding of histone H1 cannot be tested by the approach used for short fibers. The sharp decrease of sedimentation velocity (Fig. 4), however, and the finding of a similar transition of histone H1 complexes made with large DNA fragments (12) leads us to believe that histone H1 is also bound cooperatively on large fiber fragments and most likely also on native fibers at high ionic strength. The binding sites of histone H1 could be DNA sequences rich in A+T (20), because model studies showed a selective histone H1 interaction with DNA species of high A+T content (21).

Above the transition (at higher ionic strength) chromosome fibers appear to be composed of tandem arrays of globular structures with a diameter of 200 Å. The involvement of histone

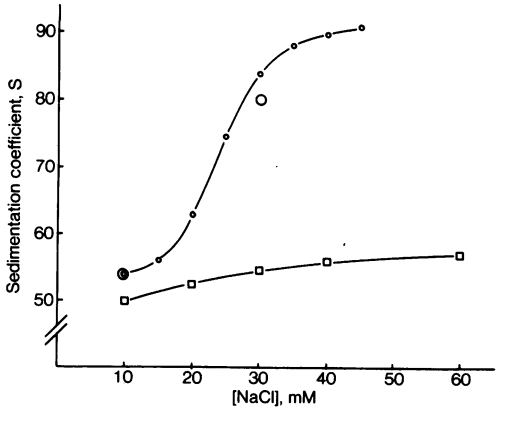

FIG. 4. Average sedimentation coefficient of large chromosome fiber fragments (average length of about 30 nucleosomes) at different NaCl concentrations. Nuclei were digested with 2 units of micrococcal nuclease per $1 \cdot 10^6$ nuclei for 2 min at 0°. After lysis and low-speed centrifugation 50% of total chromatin was in the supernatant. Aliquots (5 A_{260} units in 0.5 ml) were dialyzed against 1 mM sodium phosphate (pH 6.8), 0.2 mM EDTA, and different NaCl concentrations between 60 and 10 mM. The samples were sedimented through sucrose gradients in the same buffers as the samples at 15,400 rpm for 15 hr. The average sedimentation coefficients (○) relative to a 28S marker (mouse rRNA) are plotted against the NaCl concentration. (□) Histone H1 was removed after the micrococcal nuclease digest and the samples were dialyzed to the appropriate buffer. (◯) Histone H1 (20% by weight relative to DNA) was added to H1-depleted fiber fragments.

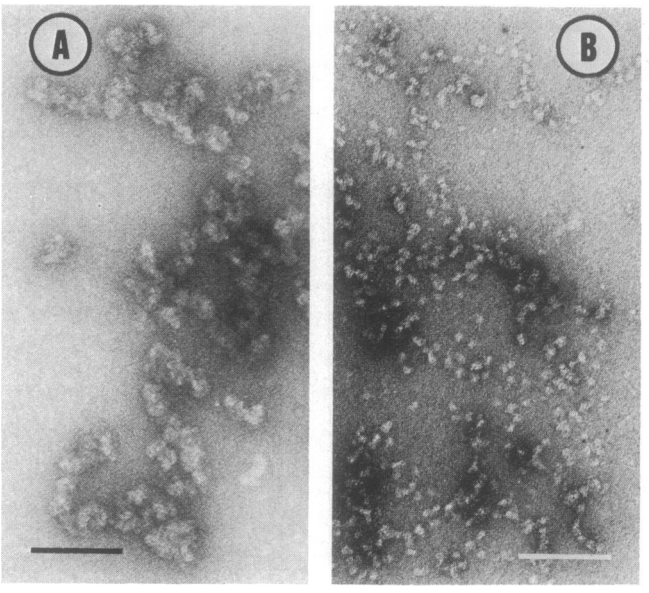

FIG. 5. Electron micrographs of chromosome fibers above (70 mM NaCl, *A*) and below (10 mM NaCl, *B*) the structural transition range. Fibers were fixed with glutaraldehyde in high and low salt, attached to carbon-coated electron microscope grids, and negatively stained with uranyl acetate. Scale lines represent 1000 Å.

H1 in the maintenance of the thick fiber seems to be unambiguous. Evidence for the discontinuity of the 200 Å fiber is bolstered by the successful enrichment of globular 200 Å structures followed controlled nuclease fragmentation of chromatin in nuclei and their extensive characterization by electron microscopy.* On the other hand there have been numerous experimental studies on chromatin, mainly by x-ray diffraction (22, 23) but also by neutron scattering (24) and electron microscopy (25, 26), that led to interpretations suggesting a helical arrangement of nucleosomes. The apparent contradiction between our conclusions and those derived by previous physical structure investigations probably results from differences in the preparation of chromatin with respect to the ionic strength of the buffers used.

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