We have examined the gel profiles of staphylococcal nuclease digests of intact nuclei following different extents of removal of histone H1 by low pH. It was found that the submonomer fragment pattern (i.e., fragments <140 base pairs (bp)) changed dramatically following removal of H1. The most striking feature of this change was a marked increase in the relative intensity of a band migrating at 102 ± 4 bp when about 20–50% of the nuclear DNA is rendered acid soluble. All other submonomer bands decreased in relative intensity. There was no evidence for an ~100-bp repeat pattern accompanying the enhanced generation of the 102-bp fragment following H1 removal. This result, along with the comparisons of gel profiles for different extents of digestion, suggests that removal of histone H1 from nuclei results in an increased susceptibility of the DNA to staphylococcal nuclease at one or both ends of many of the core particles and that a strong block to further digestion occurs within these core particles resulting in the formation of a relatively stable 102-bp fragment.

Until recently, histone H1 was thought to be associated primarily with linker regions in chromatin (see Ref. 1 for a recent review of chromatin structure). However, a number of recent studies suggest that histone H1 may also interact with at least a portion of the nucleosome core (2–5). Indeed, Simp- son (4) and Thoma et al. (5) have provided evidence suggesting that H1 both stabilizes and protects the DNA at the points of entry and exit from the core region of the nucleosome. Furthermore, chemical cross-linking studies have demonstrated contacts between H1 and core histones (6–8) as well as core DNA (9).

In the present report, we have examined the role of histone H1 in the staphylococcal nuclease digestion of nucleosomal DNA in intact nuclei and nuclei in which H1 has been selectively extracted (10). We find that removal of histone H1 from nuclei alters the digestion of nucleosome core DNA by staphylococcal nuclease and results in the formation of a relatively stable 102-bp fragment.

Removal of Histone H1 from Intact Nuclei Alters the Digestion of Nucleosome Core DNA by Staphylococcal Nuclease*

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MATERIALS AND METHODS

Nuclei Preparation and H1 Removal—Nuclei were prepared from confluent, human diploid fibroblasts (line IMR-90, See ref. 11) labeled during the growth phase with 0.025 μCi/ml of [3H]dThd (see Ref. 12 for details). The procedures for nuclei isolation and extraction of histone H1 in low pH buffers were exactly as described in Lawson and Cole (10). All buffers contained 0.1 mM phenylmethylsulfonyl fluoride. We note that during and after the low pH extraction the nuclei were much more delicate, as assessed by phase contrast microscopy, and care should be taken not to adhere rigidly to the conditions of Lawson and Cole (10) i.e. use gentle mixing of nuclei in wash buffers and low speed centrifugation (300 × g) for short times (2 min).

Nuclease Digestion—Nuclei (~7 × 10^6/ml), suspended in 50 mM Tris (pH 7.5), 25 mM KCl, 1 mM CaCl2, 1 mM MgCl2, and 0.25 M sucrose (10), were incubated at 37°C with staphylococcal nuclease (Worthington) at concentrations given in the figure legends. Digestions were carried out in 0.1 M Tris (pH 7.5), 25 mM KCl, 1 mM CaCl2, 1 mM MgCl2, and 0.25 M sucrose for 10 min.

FIG. 1. Sodium dodecyl sulfate gel profiles of retained nuclear proteins following extraction in acidic buffers. Human fibroblast nuclei were extracted in citric acid-phosphate buffer (pH as indicated in the figure) using the method of Lawson and Cole (10). The retained nuclear proteins were extracted in 0.4 M H4SO4, dialyzed, and lyophilized. Samples (10 μg) were electrophoresed on an 18% polyacrylamide slab gel containing sodium dodecyl sulfate (13). The gel was stained with Coomassie brilliant blue (R-250) and after destaining, scanned at 560 nm. The positions of the individual histones are designated in the top profile along with the positions of (presumably) nonhistone protein bands (unlabeled arrows) that were visible. Migration is from left to right.

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‡The abbreviation used is: bp, base pairs.
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Electrophoresis—Displaced and retained nuclear proteins were prepared and analyzed by electrophoresis as described by Lawson and Cole (10) with the exception that the sodium dodecyl sulfate slab gels were modified as described by Thomas and Kornberg (13). Values of the fraction of retained histone H1 given in the text were determined from the gel scans (e.g. Fig. 1) where the ratio of the peak area for H1 and the total peak area of the core histones at a given pH was compared to the value at pH 7.5.

DNA samples were prepared from nuclei as described previously (12). Electrophoresis was carried out on either 2.8% agarose slab gels (12) or 6% polyacrylamide slab gels (14). Gels were stained in 2 μg/ml of ethidium bromide, illuminated (Ultra-Violet Products, Inc., transilluminator), and photographed through a red filter with Polaroid type 55 film. Scans of the negatives were made on a Cilford spectrophotometer equipped with a linear transport device. The sizes of staphylococcal nuclease digestion products were determined from calibration of the gels with Hinfl restriction fragments of φX174 HF DNA (prepared in this laboratory by Dr. Steven Dresler).

RESULTS

Fig. 1 shows gel profiles of the retained, acid-soluble nuclear proteins from human diploid fibroblast nuclei following extraction by acidic buffers as described by Lawson and Cole (10). Most of the histone H1 is retained by these nuclei following extraction at a pH ≥3.0, whereas ∼90% of the H1 is removed at pH 2.7. This transition occurs at slightly lower pH values than was observed for HeLa nuclei (10).

Nuclei containing varying amounts of histone H1 were prepared by this method and digested to different extents with staphylococcal nuclease. The resulting DNA fragments were analyzed on 2.8% agarose gels and 6% polyacrylamide gels. We observed an ∼3-fold increase in the rate of release of nucleosome cores from H1-depleted nuclei (>90% H1 removed) compared to that of control (undepleted) nuclei (data not shown). In addition, there was no measurable change in nucleosome repeat length following removal of varying amounts of H1 (data not shown). These results are in complete agreement with those of previous reports (10, 15, 16).

Following extensive digestion, we observed differences in the submonomer fragment patterns (i.e. fragments <140 bp in length) between control nuclei and those which were partially or totally depleted of histone H1. As seen in Fig. 2A, the relative intensities of the submonomer fragments at varying digestion times were terminated with EDTA and the fraction of acid-soluble DNA was determined as described previously (12).

Restriction fragments of φX174 RF DNA with the 100- and 140-bp fragments designated by arrows. We note that the migration of samples near the edges of the gel is slightly retarded relative to that of the center lanes. B, scans of the negative for A for the 80-min digestion times (lanes 6, 11, and 13) and the Hinfl φX174 RF marker fragments (bottom profile). The sizes (in base pairs) of the smaller restriction fragments are given below the bottom profile. The sizes (in base pairs) determined for the larger submonomer fragments are given in the top profile. We note that the 61-bp submonomer "peak" actually corresponds to a doublet of 2 closely migrating bands.
extents of digestion differ for the three different pH conditions used. These gels correspond to nuclei having 0–5% (pH 3.25), 40–60% (pH 2.85) and 85–95% (pH 2.70) of the total histone H1 removed (Fig. 1). A striking feature in the patterns shown is the marked increase in relative intensity of the band migrating at ~100 bp following H1 removal. (The actual size of this fragment was determined to be 102 ± 4 bp.) This increased intensity is seen more clearly in Fig. 2B which shows scans of the gel lanes corresponding to ~90% of the nuclear DNA rendered acid soluble in each case. These data also reveal that for these extents of digestion essentially all of the discrete submonomer fragments shown decrease in their intensity relative to those at 102 and 142 bp when H1 is removed.

Fig. 3 shows the gel profiles for different extents of digestion of nuclei extracted at pH 7.5, 3.25, 2.7, and 2.5. As can be seen, there is very little difference between the profiles for nuclei that have been treated with pH 3.25 buffer and those for the control nuclei (i.e. nuclei extracted with pH 7.5 buffer). Also, following either ~90% (pH 2.7) or ~100% (pH 2.5) removal of histone H1, the band at 102 bp increases in relative intensity even when primarily nucleosome monomers and submonomer fragments are present. This result, along with the fact that we did not observe the 102-bp fragment at very early times of digestion (data not shown, however, see Fig. 2A), suggests that this band is derived from nucleosome core DNA.

**DISCUSSION**

We have shown that the removal of histone H1 from intact nuclei by low pH results in a change in the submonomer fragment pattern generated by extensive digestion with staphylococcal nuclease. This change involves an increase in the relative intensity of the band migrating at 102 ± 4 bp and a decrease in the relative intensity of all other submonomer fragments over the range where about 20–50% of the DNA is rendered acid soluble.

The marked increase in relative intensity of the 102-bp fragment following H1 removal could arise from 1) an increase in nonspecific exonucleolytic “trimming” of the DNA at one or both of the ends of the core particle, 2) an increase in the relative cutting frequency at specific sites in the DNA at one or both ends of the core particle, or 3) an increase in the relative cutting frequency at a specific site (or set of sites) in the DNA near the center of the core particle. Careful examination of our data, as well as the results of others, leads us to favor the second possibility. As can be seen in Figs. 2 and 3, at early digestion times, the 102-bp peak appears to arise from larger, discrete fragments being successively cleaved to the 102-bp size. Furthermore, we do not observe an ~100-bp repeat pattern superimposed on the normal ~200-bp pattern (Fig. 2A). This 100-bp repeat pattern would be expected if a specific site near the center of the core DNA were being exposed by H1 removal. Indeed, such a pattern is observed when chromatin is digested with DNAase II in the presence of divalent cations (17, 18). (It is interesting to note that removal of H1 markedly reduces the relative cutting frequency at the intracore site by DNase II.) We wish to point out, however, that although these enzymes must use different modes of attack on core DNA, it is still possible that the ~100-bp fragments generated by staphylococcal nuclease and DNase II arise from the same region of the core particle.

In a related study, Riley and Weintraub (19) used exonuclease III to digest chromatin. These authors found that this enzyme digests nucleosome core DNA from both 3' ends and generates a series of discrete fragments down to a size of ~100 bases. At this point, there is a distinct pause in the digestion. It is interesting to speculate that in the absence of H1 staphylococcal nuclease also readily digests the ~20 bp of DNA at the ends of the core particle and is then confronted with the same constraints as exonuclease III to further digestion. We also note that thermal denaturation studies on isolated core particles (lacking histone H1) (20–22) and studies on the availability of regions of the core particle DNA to DNase I (21, 23–25) are consistent with the view that in the absence of H1 the DNA at the ends of the core particle is less tightly constrained than the “inner” ~100-bp region of DNA.

Further examination of the profiles in Figs. 2 and 3 reveals that not all of the core DNA is readily digested to the 102-bp fragment. The DNA in a significant fraction of the core particles is much more resistant to staphylococcal nuclease digestion and appears to be degraded to fragments other than just the 102-bp size (e.g. Fig. 3, L and P). Thus, not all nucleosome cores are digested in the same way by staphylo-

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2 Also, M. J. Smerdon and M. W. Lieberman, unpublished results.
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It appears that the digestion characteristics of a subfraction of the nucleosome core population are either unaffected by H1 removal or altered in a different way than those giving rise to the stable 102-bp fragment.

Finally, we wish to address the possibility that exposure of nuclei to low pH causes an irreversible conformational change in the nucleosome structure that gives rise to an altered submonomer pattern following staphylococcal nuclease digestion. Indeed, Zama et al. (26) have clearly demonstrated such a change in isolated nucleosomes. Although one can argue that the pH-induced transitions observed by these authors may not apply to intact nuclei on the basis that nucleosome structure may be much more constrained within whole chromatin, a more pleasing argument can be made from consideration of actual data. First, the change in the submonomer pattern that we observe correlates well with the removal of histone H1. Second, Zama et al. (26) observed the irreversible changes in nucleosome monomers to occur between pH 4.2 and 4.6, whereas the changes that we observe are between 2.5 and 3.0. Third, data in the literature suggest that removal of histone H1 by methods that do not involve exposure to low pH results in a change in the submonomer pattern similar to what we observe. Fig. 4 of Camerini-Otero et al. (27) shows staphylococcal nuclease digestion patterns for duck erythrocyte chromatin depleted of histones H1 and H5 by exposure to tRNA at neutral pH (28). The data clearly demonstrate a marked enhancement in the relative intensity of the 100-bp fragment compared to the digestion patterns of nondepleted (control) chromatin (Figs. 1, 2, and 4 of that reference). Furthermore, Muyldermans et al. (29) observed the appearance of a prominent band (“band X”) in the submonomer region of the gel following staphylococcal nuclease digestion of H1-depleted chicken erythrocyte chromatin, whereas this band was much weaker in intensity in undepleted chromatin (see Fig. 6 of that reference). These authors used an ion exchange resin at neutral pH to selectively remove histone H1. Thus, it is unlikely that our observations are the result of an irreversible conformational change in nucleosome structure induced by extraction at low pH.

REFERENCES