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SENSITIVITY OF REGIONS OF CHROMATIN CONTAINING HYPERACETYLATED HISTONES TO DNASE I

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SUMMARY

We have used DNase I as a probe for structural changes in regions of chromatin containing highly acetylated histones. The enzyme preferentially digests regions of chromatin that are associated with rapidly or highly acetylated histones, suggesting that nucleosomes in these regions are in a more accessible conformation. This sensitivity to DNase I may be related to the same factors which cause the differential digestion of active genes.

INTRODUCTION

Histones are organized along the DNA in eukaryotes to form nucleosomes, consisting of a compact core containing two copies each of histone H2a, H2b, H3 and H4 associated with 140 base pairs of DNA (1-3) together with a more flexible spacer region of 40 to 60 base pairs of DNA associated with the H1 histones (4,5). An analysis of the reassociation kinetics of DNA isolated from nucleosomes indicates that this structure is present on both template active and inactive regions of the genome (6-8). Because histone-histone and histone-DNA interactions within the nucleosome tightly fold the DNA, it seems unavoidable but that it must be necessary to either completely unfold or partially release the DNA to allow polymerases to move through this structure. Subsequently it would be necessary to refold the nucleosome or regenerate histone-DNA interactions as rapidly as possible. Since the histones are actively modified in all cells, histone modification is one of the more likely candidates for weakening or strengthening histone-histone or histone-DNA interactions.

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Of the various sorts of histone modification, histone acetylation appears to be the most likely candidate for weakening and regenerating interactions in the nucleosome since 1) histone acetylation takes place very rapidly and likewise turns over vigorously showing half-lives of less than 3 minutes (9), 2) acetylation occurs on the "inner" or more tightly bound histones presumably causing greater changes in nucleosome structure and 3) this modification is found on the lysine residues in the N-terminal region of the histones. This is the region of the histone involved in binding DNA, and thus acetylation might be expected to affect weaker ionic interactions between histone and DNA.

Transcriptionally active globin, ovalbumin and adenovirus genes are preferentially digested by DNase I (10-12) presumably due to an aspect of nucleosome organization which makes the DNA more accessible to this nuclease. Accordingly we have digested chromatin with DNase I to determine if this enzyme preferentially digests regions of the genome containing hyperacetylated histones. We find that DNase I is sensitive to regions of the genome containing rapidly or highly acetylated histones and these regions are preferentially degraded to nucleohistone fragments that are on the average, smaller than those of the bulk of the digested chromatin.

MATERIALS AND METHODS

Hepatoma tissue culture (HTC) cells were grown in suspension culture as described by Oliver et al. (13). For [^{3}H] acetate labelling (5 mCi/l), cells in log phase (4-6 x 10^5 cells/ml) were labelled for 1 hr before isolation.

Nuclei were isolated by two washes in 0.25 M sucrose, 60 mM KCl, 15 mM NaCl, 10 mM $MgCl_2$, 1 mM $CaCl_2$, 6 mM Na-butyrate, 10 mM MES (2[N-morpholino] ethane sulfonic acid) pH 6.5 plus Triton X-100 and digested in the same buffer without Triton X-100. Digestion of nuclei at 37° was with DNase I (1 unit/50 µg DNA) or micrococcal nuclease (5 units/50 µg DNA). Acid solubility was determined as described by Garel and Axel (11).

Acid extraction of histones, electrophoresis, staining and destaining of 23 cm acid-urea gels (2 M urea) are as described by Panyim and Chalkley (14). 2 mM slices from acid-urea gels were digested with 0.3 ml 30% hydrogen peroxide and counted in Bray's solution. Nucleohistone fragments were electrophoresed at 130 volts for 1.5 hr on 9 cm, 6% polyacrylamide gels containing 3 M urea, 10 mM MES, 6 mM Na-butyrate, 2 mM EDTA pH 6.5 tris. Digested samples were dialyzed into the same buffer before electrophoresis. The tray buffer was as above, but without urea. Fragments from the nucleohistone gels were cut out, incubated in 1% SDS, 1% 2-mercaptoethanol, 10 mM tris, 6 mM Na-butyrate, 5 mM EDTA, pH 7.0 and placed directly on SDS histone (15,16) and DNA gels (17) containing 0.1% SDS.

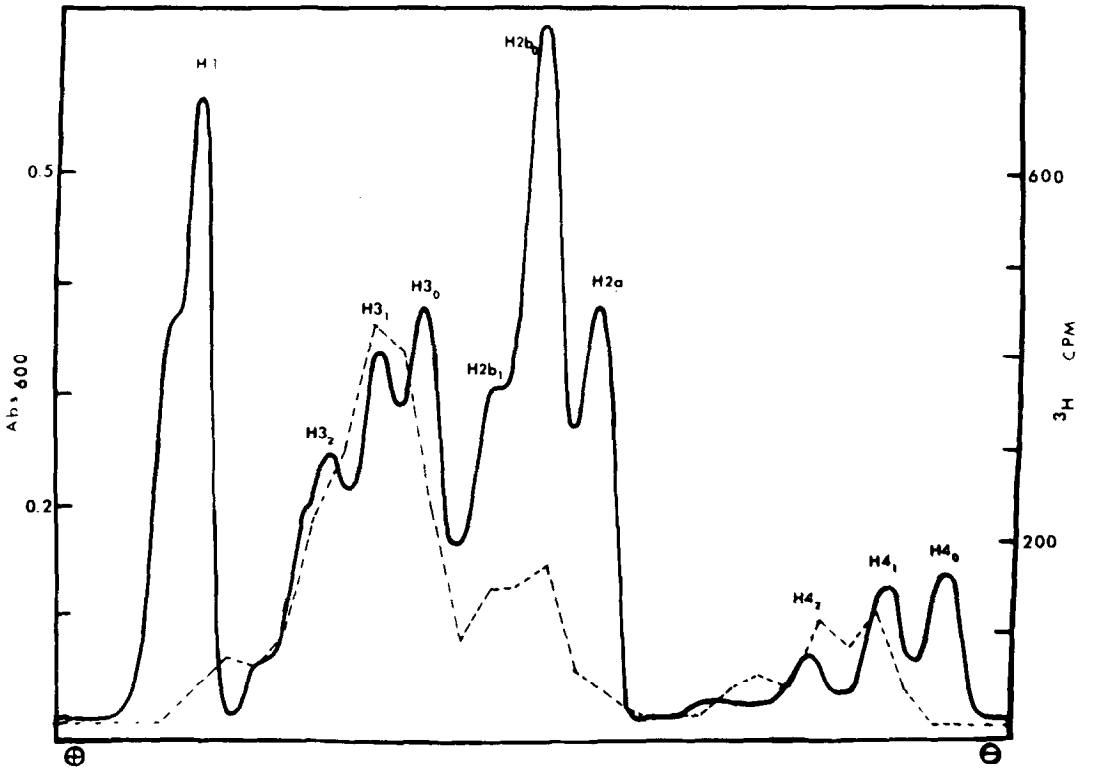

Figure 1. Incorporation of [^{3}H] acetate into Hepatoma Tissue Culture (HTC) cell histones. The gel profile (solid line) was obtained by scanning (600 nm) an amido black stained 23 cm acid-urea (2 M) gel that was loaded with 60 µg of HTC histone and electrophoresed for 48 hr at 200 volts. Two millimeter gel slices were treated with hydrogen peroxide and counted in Bray's solution. Counts per minute profile - dashed line. H_0 = parent histone. $H_{1,2}$ = mono and di-acetylated forms.

RESULTS AND DISCUSSION

HTC histones labelled with [^{3}H] acetate were electrophoresed on long acid-urea gels to separate the modified forms of each of the histone classes. Figure 1 demonstrates that only histone bands that are known to contain the acetylated species incorporate [^{3}H] acetate during the 1 hr labelling period. H3, H3b, H2a and H4 all become labelled in bands moving more slowly than the parental band. Further, neither Hl nor the parent forms of H2a and H4 contain label after short pulses. Thus the vast majority of the [^{3}H] label associated with a given histone reflects modification of the histone by acetylation and

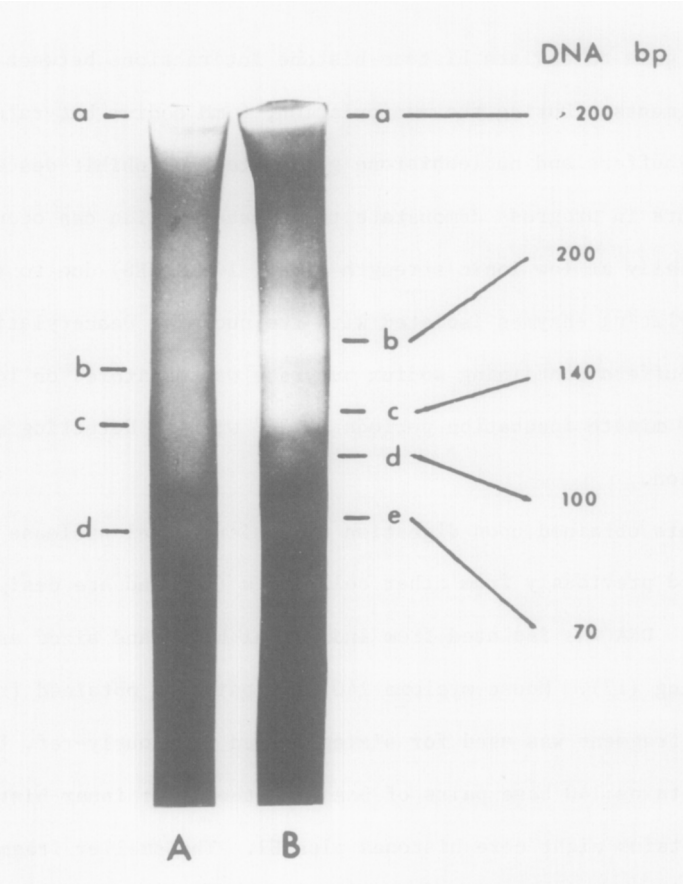

Figure 2. Nucleohistone fragments from nuclease digested HTC nuclei, electrophoresed on 6% polyacrylamide gels containing 3 M urea, 10 mM MES, 2 mM EDTA, pH 6.5 (tris). (A) Fragments (a-d) obtained after digestion with DNase I to 7% acid solubility (5 min. digest). (B) Fragments (a-e) obtained after micrococcal nuclease digestion to 35% acid solubility (20 min. digest). Right - corresponding DNA sizes.

we conclude that radiolabel associated with a given band in an SDS gel will represent modification by acetylation even though in this system individual modified species do not migrate separately.

Nuclei from [^{3}H] acetate-labelled cells were digested to 7% acid solubility with DNase I. For comparison, an aliquot of the same nuclei was digested to 35% acid solubility with micrococcal nuclease since this enzyme shows no preference for active gene regions after exhastive digestion (10). The fragments were electrophoresed on 6% polyacrylamide gels (figure 2), urea was

included in the gels to obviate histone-histone interactions between the subnucleosomal fragments. During the manipulation, 6 mM sodium butyrate was included in the buffers and nucleohistone gel system to inhibit deacetylation (18). Experiments in progress demonstate that deaceytlation can occur rapidly in vitro, especially at low ionic strength (i.e., 10 mM MES) due to the presence of deacetylating enzymes isolated with the nuclei. Deacetylation in the various buffers containing sodium butyrate was monitored on long acid-urea gels for 60 minute incubation periods at 37° without detecting significant deacetylation.

The fragments obtained upon digestion with micrococcal nuclease correspond to those obtained previously from other cell types (19) and are designated (a) through (e). DNA was isolated from individual bands and sized as described by Loening (17). Mouse myeloma 140 base pair DNA obtained from the nucleosome core fragment was used for sizing (sized previously-ref. 19). Fragment (c) contains 140 base pairs of DNA plus the eight inner histones, fragment (b) contains eight core histones plus H1. The smaller fragments (d and e) are obtained from digestion within the nucleosome core. Fragments from the DNase I digest are labelled (a) through (d), and the mobilities of fragments from both digests are given in TABLE I. An unusual and as yet unexplained result of the DNase I digest is that although the fragments contain similar DNA sizes, they appear to be depleted in H2a and H2b. This is currently under investigation.

The bands were cut out of the nucleohistone gels, treated with SDS to dissociate the histone, and electrophoresed onto 18% polyacrylamide SDS histone gels (figure 3). In this case all modified forms of any given histone migrate with the parental species allowing accurate determination of the specific activities of each histone class taken as a whole. The total counts per minute associated with H3 and H4 are shown directly in the figure as is the amount of material present for each of the four histone classes. In figure 4 the specific activites of histone H3 and H4 are plotted versus the

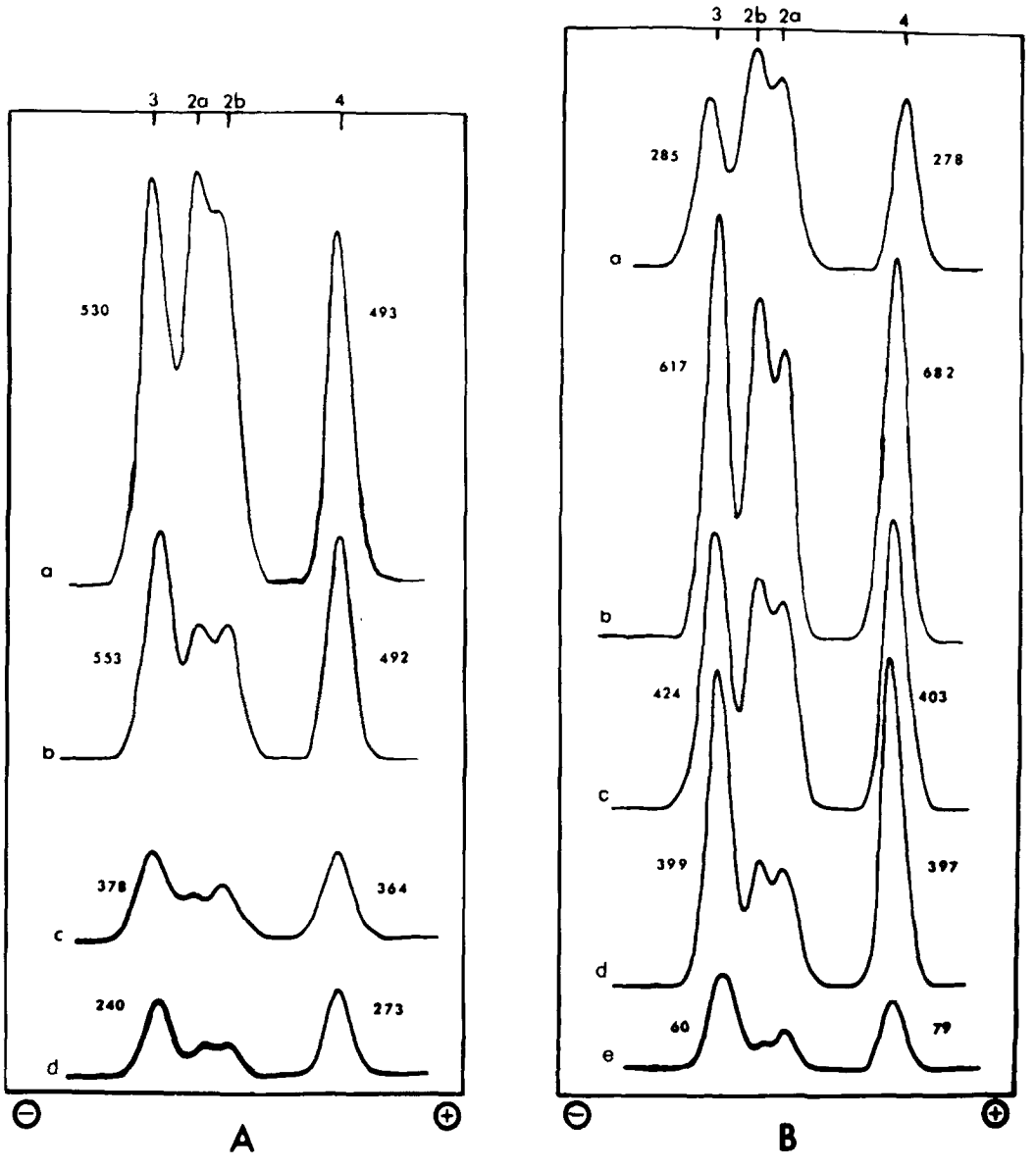

Figure 3. Gel scans of histones from nucleohistone fragments, electrophoresed on 16 cm, 18% polyacrylamide SDS gels. Gels were stained with amido black and scanned at 600 nm (H1 region is not shown). (A) From DNase I digest, fragments a-d, and (B) from micrococcal nuclease digest, fragments a-e. The [^{3}H] acetate counts per minute are given for each H3 and H4 band.

relative mobility of the fragment from which they were obtained. The small fragments obtained upon a short DNase I digestion contain more highly acety-

TABLE I

Relative Mobilities of Nucleohistone Fragments (end of gel = 1.00)

DNase I fragments		Micrococcal nuclease fragments	
(a) Gel top	0.00	(a) Gel top	0.00
(b)	0.30	(b)	0.28
(c)	0.34	(c)	0.33
(d)	0.40	(d)	0.38
		(e)	0.44

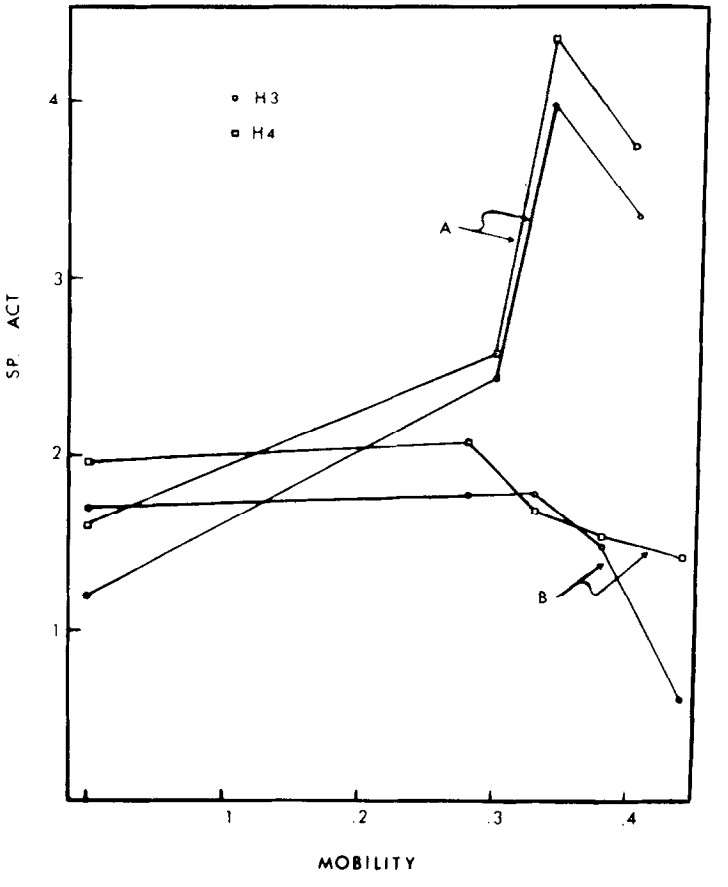

Figure 4. The specific activity (cpm/area under the curve) for H3 and H4 from each nucleohistone fragment as a function of the relative mobility of each fragment. (A) from DNase I digest, and (B) from micrococcal nuclease digest.

lated H3 and H4, or H3 and H4 with a lower acetate turnover rate as compared to these proteins obtained from the gel tops or from fragments obtained by micrococcal nuclease.

We conclude that DNase I is sensitive to regions of chromatin with high acetate incorporation in the histones. Since DNase I is able to make single-stranded nicks within the 140 base pair nucleosome core (20), the increased susceptibility of nucleosomes containing more highly acetylated histones would appear to be a manifestation of changes in nucleosome structure. Previously, DNase I has been shown to preferentially digest active regions of chromatin (8-10). Thus it is quite possible that histone acetylation changes the nucleosome structure in these active regions and this imparts to the nucleosome an increased sensitivity to nuclease digestion.

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