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A Phosphatase Inhibitor Enhances the DNase I Sensitivity of Active Chromatin†

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Received November 27, 1990; Revised Manuscript Received February 13, 1991

ABSTRACT: Although it is well-known that active domains of chromatin have elevated DNase I sensitivity, it can be difficult to observe preferential sensitivity in many cell types. We show that the DNase I sensitivity of active chromatin is enhanced some 10-fold by treating nuclei with the phosphatase inhibitor *p*-(chloromercuri)benzenesulfonic acid (CMBS) whereas DNase I sensitivity in inactive domains is only 3-fold higher. We further show that CMBS-enhanced DNase I sensitivity is associated with at least two histone modifications. First, the negatively charged CMBS molecule becomes covalently attached to the thiol groups on histone H3. Second, histone H2A phosphorylation is significantly elevated in treated nuclei. The phosphorylation data along with other results point to the possibility that H2A phosphorylaation plays a role in enhancing preferential DNase I sensitivity. Whatever the mechanism, CMBS treatment of nuclei followed by DNase I digestion provides a novel and reproducible assay for probing the chromatin structure of active domains.

Pancreatic DNase I detects two structural features that are characteristic of active chromatin. First, there are the short 100–400 bp openings in chromatin, the DNase I hypersensitive sites (Gross & Garrard, 1988), which often indicate binding sites for transcription factors. The second structural characteristic of active chromatin is domain DNase I sensitivity (Weintraub & Groudine, 1976; Stalder et al., 1980; Wood & Felsenfeld, 1982), which extends over many kilobases of chromatin and encompasses nontranscribed as well as transcribed DNA sequences (lawson et al., 1982; Alevy et al., 1984; Jantzen et al., 1986). While little is known about the structural features responsible for domain DNase I sensitivity, it is thought to render a chromatin domain accessible to regulatory factors and may be an obligatory step in the initiation of RNA transcription (Weintraub, 1985).

While DNase I hypersensitivity is readily detectable in all eukaryotic cells, current procedures for detecting domain DNase I sensitivity work poorly, if at all, for many cell types. We reasoned that endogenous nucleases, proteases, or phosphatases may degrade nuclear preparations and dampen the differences between active and inactive domains. Therefore, we searched for a broad-based inhibitor of degradative enzymes which would allow the reproducible detection of domain DNase I sensitivity in all cell types.

Several sulfhydryl reagents have been reported to inhibit dephosphorylation and proteolysis of histones (Paulson, 1980). In this report, we show that the irreversible sulfhydryl reagent *p*-(chloromercuri)benzenesulfonic acid (CMBS)[1] induces a dramatic increase in DNase I sensitivity in active chromatin, whereas inactive chromatin is less affected. We also explore the possible mechanisms of CMBS-enhanced DNase I sensitivity.

Experimental Procedures

Recombinant DNA. The full-length RB clone [p4.7R cDNA described in Friend et al. (1986)] was obtained from the Massachusetts Eye and Ear Infirmary. Plasmids containing the β-actin and AS coding regions (Beaudet et al., 1986) were obtained from Svend Freytag.

Cell Culture, Nuclear Isolation, and DNase I Digestion. RPMI 2650 cells (transformed nasal septum cells, ATCC CCL 30), Wilm's tumor cells (ATCC CRL 1441), and ductal

† Supported by grants from the American Cancer Society (CD-385) and the National Science Foundation (DMB-9018701).
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[1] Abbreviations: CMBS (CMPS in figures), *p*-(chloromercuri)-benzenesulfonic acid; IAA, iodoacetamide; DEPC, diethyl pyrocarbonate; AS, argininosuccinate synthetase; RB, retinoblastoma; RSB, nuclear isolation buffer (10 mM Tris-HCl, 10 mM NaCl, and 3 mM $MgCl_2$); kb, kilobase(s).

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breast carcinoma cells (ATCC HTB 129) were grown in monolayer cultures in a 1:1 mixture of Dulbecco's modified Eagle's medium and Ham's nutrient F-12 media (Sigma, 8900) supplemented with 10% bovine calf serum (Hazelton Laboratories). The cells were pelleted and resuspended in cold RSB buffer with and without sulfhydryl inhibitors. For experiments with the CMBS inhibitor, CMBS (Sigma) was dissolved in 0.5 M Tris-HCl (pH 8.0) and diluted a thousandfold to a final concentration of 1 mM in RSB buffer. After 5 min in RSB buffer, 1% NP40 was added to lyse the cells, and the lysate was centrifuged at 2000*g* for 5 min to pellet nuclei. The nuclei were resuspended in RSB without inhibitors at a concentration of 16 OD_{260} units/mL (assayed by dilution into 2 M NaCl and 5 M urea) and stored on ice briefly. Each sample was split into five fractions containing 5.7 A_{260} units of nuclei in 370 μL, and the fractions were preincubated at 37 °C for 5 min. The nuclei were digested for 3 min at 37 °C with 0, 0.11, 0.33, 1, or 3 μg of DNase I (Sigma, D-5025). Reactions were stopped by adding SDS and EDTA to a final concentration of 0.4% SDS and 20 mM EDTA.

DNA Isolation, Southern Blotting, Hybridization, and Autoradiography. DNA was purified from the nuclei as previously described (Villeponteau & Martinson, 1981) and digested overnight at 37 °C with 2 units of various restriction enzymes per microgram of DNA. The restricted DNA was fractionated on 1% agarose gels and transferred to nitrocellulose by Southern blotting (Southern, 1975). Blot hybridization to α-^{32}P-labeled probes and autoradiography at −80 °C for 5–10 days were carried out as described previously (Villeponteau & Martinson, 1981).

Histone Extraction. Histones were prepared by extracting the nuclei in 0.2 M H_2SO_4 at 4 °C for 4 h, pelleting in the microfuge for 15 min at 4 °C to remove nuclear debris, and precipitating the histones with 3 volumes of ethanol (−20 °C, 12 h). Total histone concentration was determined by measuring the absorbance at 230 nm. Histones were resuspended in 1 mM HCl at 1 mg/mL.

Electrophoretic Analysis. High-resolution acetic acid–urea/polyacrylamide gels (Hardison & Chalkley, 1978) consisting of 2.5 M urea, 0.9 M acetic acid, and 15% polyacrylamide were cast in 32-cm plates. The gels were preelectrophoresed in an electrode buffer of 2.5 M urea/0.9 M acetic acid for 24 h at 100 V. Fifty micrograms of histone was loaded per lane at a concentration of 1 mg/mL in 2.5 M urea/0.9 M acetic acid and was run in 0.9 M acetic acid at 250 V for 36 h. When electrophoresis was complete, the gels were fixed in 12.5% trichloroacetic acid and stained with Coomassie blue (Sigma). After being stained, the gels were dried between cellulose sheets for photography.

Results

CMBS Dramatically Alters the DNase I Sensitivity of Bulk Chromatin. Previous work indicated that nuclei isolated in the presence of the sulfhydryl reagent CMBS have altered kinetics of DNase I digestion as detected by the more rapid release of acid-soluble nucleotides (Prentice & Gurley, 1983). To determine the effects of various sulfhydryl reagents on DNase I sensitivity, we isolated nuclei from human RPMI cells in the presence or absence of IAA or CMBS. The nuclei were then digested for 3 min with increasing concentrations of DNase I, and the digestion products were electrophoresed on agarose gels. The ethidium bromide stained gel (Figure 1A) demonstrates that IAA and control nuclei are resistant to all but the highest of DNase I (3 μg in lane 4, 9 μg in lane 5), at which point the nuclear DNA is rapidly digested. A similar

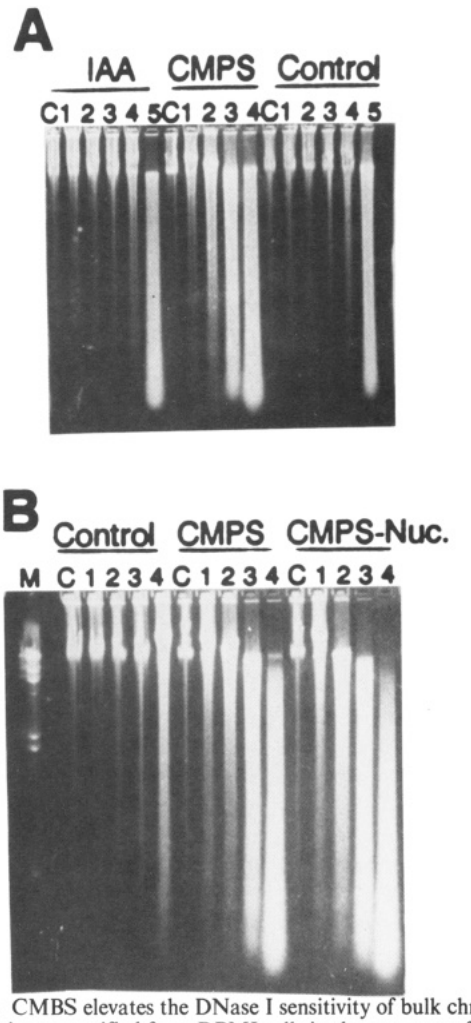

FIGURE 1: CMBS elevates the DNase I sensitivity of bulk chromatin. (A) Nuclei were purified from RPMI cells in the presence of 10 mM iodoacetamide (IAA) or 1 mM CMBS or in the absence of inhibitor (control). (B) Nuclei were purified in the presence or absence of CMBS. Control nuclei purified in the absence of CMBS were split onto two aliquots, and one aliquot of control nuclei was treated with CMBS (CMPS-Nuc.). All nuclear samples were subjected to increasing concentrations of DNase I (lanes 1–5 containing 0.11, 0.33, 1.0, 3.0, and 9.0 μg, respectively, in 400 μL of buffer). DNA was isolated and electrophoresed on 1% agarose gels. Lanes C contain undigested DNA from each nuclear sample. Lane M in (B) contains *Hin*dIII-cut λ.

pattern of digestion was seen with nuclei purified in the presence of the sulfhydryl reagent 5,5′-dithiobis(2-nitrobenzoic acid) (data not shown). In contrast, nuclei isolated in the presence of CMBS (middle panels of Figure 1) are degraded over a broad range of DNase I concentrations beginning with the lowest levels of 0.11 μg of DNase I in lanes 1. This same enhanced and broadened pattern of DNase I sensitivity with CMBS treatment has been observed in HeLa cells, human breast tumor cells, Wilm's tumor cells, human fibrosarcoma HT-1080 cells, and mouse L cells (data not shown). Control DNase I experiments with naked DNA and CMBS show that CMBS slightly inhibits DNase I activity (data not shown), so that the increases in DNase I sensitivity seen in CMBS-treated nuclei cannot be caused by a CMBS-induced stimulation of the DNase I enzyme. We conclude from these data that the isolation of nuclei in the presence of CMBS leads to an increased DNase I sensitivity of at least a fraction of the chromatin in nuclei of various cell types.

The elevated DNase I sensitivity of nuclei isolated in the presence of CMBS might be caused by the inhibition of degradative enzymes transiently present during nuclear iso-

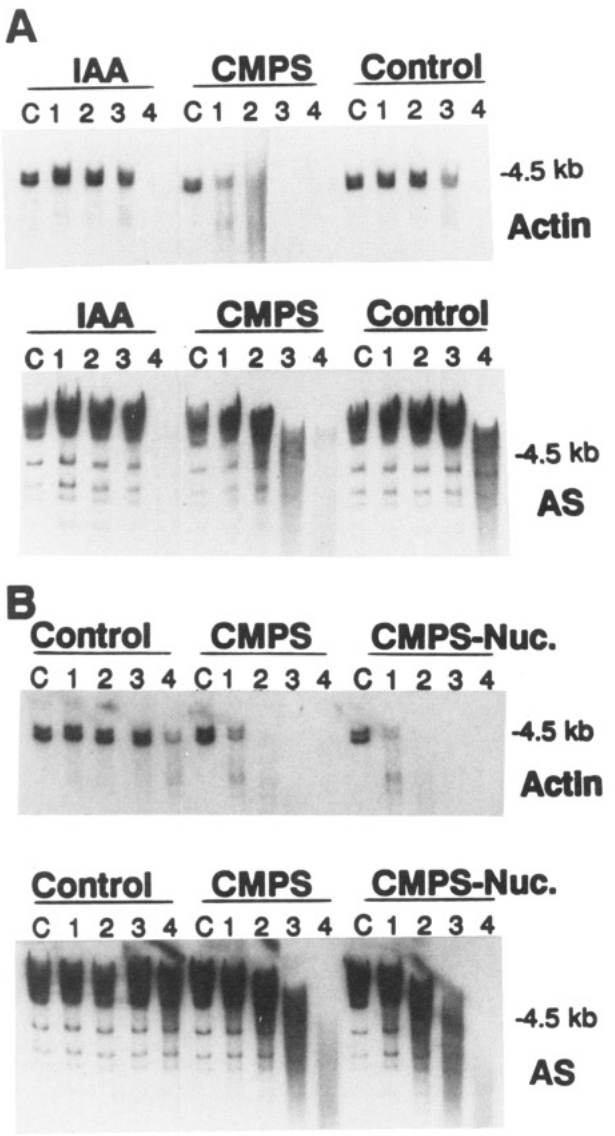

FIGURE 2: CMBS enhances the preferential sensitivity of β-actin. The DNA samples in Figure 1 were restricted with *Bam*HI, run on two 1% agarose gels, and Southern-blotted. Each blot was first hybridized to an actin cDNA probe and autoradiographed (upper blots in panels A and B). The blots were then washed to remove the actin probe and rehybridized to AS (lower blots).

lation or by some change in the proteins of the final nuclear isolates. To test which of these possibilities is correct, we treated previously isolated control nuclei with CMBS. The pattern of DNase I digestion of these treated nuclei (CMBS-Nuc) is essentially the same as nuclei isolated in the presence of CMBS (Figure 1B). Thus, we conclude that CMBS acts on the proteins present in isolated nuclei to enhance DNase I sensitivity.

CMBS Treatment of Nuclei Elevates the Preferential DNase I Sensitivity of Active Domains. The CMBS-induced elevation in DNase I sensitivity of a fraction of RPMI chromatin suggested to us that the DNase I sensitivity of active domains might be more enhanced by CMBS treatment than are inactive domains. To test for this possibility, DNA samples from the digestion experiments shown in Figure 1 were restricted with *Bam*HI, run on agarose gels, and Southern-blotted. The blots were first hybridized to a labeled probe for β-actin-coding sequences (top halves of Figure 2A,B). The blots were then washed to remove the β-actin probe and were rehybridized to the inactive AS pseudogene probe (bottom halves of Figure 2A,B).

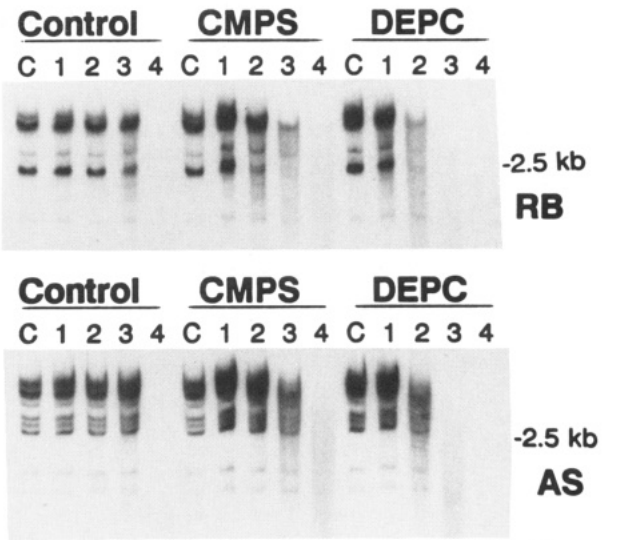

FIGURE 3: CMBS enhances the preferential sensitivity of the RB gene. Nuclei were prepared from RPMI cells in the absence of inhibitors (control) or in the presence of 1 mM CMBS or 0.1% DEPC. The nuclear samples were digested with DNase I as in Figure 1, and the DNA was purified. The DNA samples were restricted with *Eco*RI, electrophoresed, and Southern-blotted. The blot was first hybridized to a RB probe and autoradiographed (upper blot). The sensitive RB band is the one indicated at 2.5 kb. The blot was then washed to remove the RB probe and rehybridized to AS (lower blot).

Figure 2 demonstrates that β-actin is some 10-fold more sensitive to DNase I in nuclei isolated with CMBS whereas IAA has little or no effect. β-Actin gains the same 10-fold sensitivity if CMBS is added after rather than before nuclear isolation (CMPS-Nuc panel in Figure 2B). In contrast to the β-actin gene, however, the inactive AS pseudogenes are only some 3-fold more sensitive in both CMBS-treated samples (see bottom halves of Figure 1A,B). Thus, the data in Figure 2 indicate that CMBS but not IAA preferentially enhances β-actin DNase I sensitivity.

To test whether β-actin is unique in its response to CMBS, we performed a similar DNase I experiment using the active RB gene. We find that a 2.5-kb *Eco*RI band hybridizing to the RB probe has a low level of DNase I sensitivity in control nuclei (left panel of Figure 3). The 2.5-kb RB band becomes much more sensitive in nuclei isolated in the presence of CMBS (middle panel of Figure 3), indicating that CMBS-induced enhancement of DNase I sensitivity is also characteristic of this active domain.

We have also assayed the inactive β-globin gene following CMBS treatment of nuclei and found that the kinetics of β-globin digestion are indistinguishable from those found for the AS pseudogenes (data not shown). These results, along with the bulk chromatin digestion data (Figure 1), indicate that all inactive sequences have lower levels of increased DNase I sensitivity in response to CMBS treatment than active sequences.

Since CMBS enhances the DNase I sensitivity of active domains some 3-fold more than inactive regions, we wondered whether any agent that increases the DNase I sensitivity of bulk chromatin would likewise preferentially enhance the DNase I sensitivity of active sequences. To test for this possibility, we isolated nuclei in the presence of DEPC. DEPC is known to covalently bind histidyl residues in proteins (Miles, 1977), and we have shown that histones from DEPC-treated nuclei undergo mobility shifts on acid–urea gels due to the covalent binding of negatively charged DEPC molecules (data not shown). The right-hand panel of Figure 3 demonstrates that DEPC treatment elevates inactive gene (AS) DNase I

sensitivity even more than does CMBS. However, the DNase I sensitive RB fragment is degraded at the same rate as the AS pseudogenes and the DNase I insensitive RB fragments. Thus, DEPC does not enhance the preferential DNase I sensitivity of active domains although bulk, active, and inactive chromatins are all more rapidly degraded. We conclude that the CMBS-induced enhancement in preferential DNase I sensitivity is not solely the result of the increased DNase I sensitivity of bulk chromatin.

We have also checked whether CMBS treatment can enhance preferential DNase I sensitivity in normal diploid fibroblasts (MRC-5 cells) and in embryonic chick erythrocytes, in which the transcribed β-actin gene already has high preferential sensitivity. In both MRC-5 and chick erythroid cells, the DNase I sensitivity of inactive and active chromatin is increased some 3-fold (data not shown). Thus, the preferential sensitivity of active domains is not enhanced by CMBS treatment in cells which already have high levels of preferential sensitivity.

CMBS Becomes Covalently Attached to H3 Histones in Nuclei. Our observation that the digestion of bulk and inactive chromatin is elevated on treatment with CMBS raises the question of whether CMPS might directly bind histones in chromatin. Because CMBS is specific for binding sulfhydryl groups (Kenyon & Bruice, 1977), we wondered whether CMBS might react with the thiol groups of histone H3, the only histone to possess such groups. Since CMBS is negatively charged, the covalent attachment of CMBS to histone H3 should change the H3 migration on acid–urea/polyacrylamide gels. To test for CMBS binding, nuclei from four different cell lines were purified in the presence or absence of CMBS (Figure 4A). Histones were acid-extracted from all nine preparations, and the purified histones were electrophoresed on a 32-cm acid-urea/polyacrylamide gel. The Coomassie-stained gel (Figure 4A) demonstrates that CMBS-treated samples (lanes 2, 3, 5, 7, and 9) have retarded H3 migration rates in comparison to control samples (lanes 1, 4, 6, and 8). These data are consistent with the covalent attachment of the negatively charged CMBS to H3 histone thiol groups.

Since H3 phosphorylation can also account for the slowed migration of histone H3 on acid–urea gels, we have carried out three control experiments to rule out the possibility that CMBS preserves a preexisting H3 modification such as phosphorylation. First, nuclei were purified in the presence of CMBS and then incubated with alkaline phosphatase. Lane 3 in Figure 4A shows that phosphatase has no effect on the migration of H3 from RPMI nuclei exposed to CMBS. These data are not consistent with the preservation of in vivo H3 phosphorylation as the cause of the change in H3 migraation.

Second, purified histones from control nuclei never exposed to CMBS were treated with CMBS, and the reaction products were electrophoresed on acid–urea gels. Figure 4B shows the results with untreated (lane 5) and CMBS-treated (lane 6) histones. CMBS treatment of purified histones leads to the same alteration in H3 mobility as seen when CMBS is used with nuclei. This result strongly argues that CMBS directly binds to histone H3 rather than preserves some preexisting modification or interacts through some other chromosomal protein.

To verify the assumption that CMBS binds to H3 thiol groups, we pretreated control histones with IAA which is uncharged but specific for H3 thiols (Kenyon & Bruce, 1977). The IAA-treated histone was split into two aliquots which were incubated with or without CMBS. The aliquots were then run on an acid–urea gel as before. Lanes 1 and 2 in Figure 4B show that CMBS is blocked from inducing the shift in H3 histone, indicating that CMBS interacts only with H3 thiol groups.

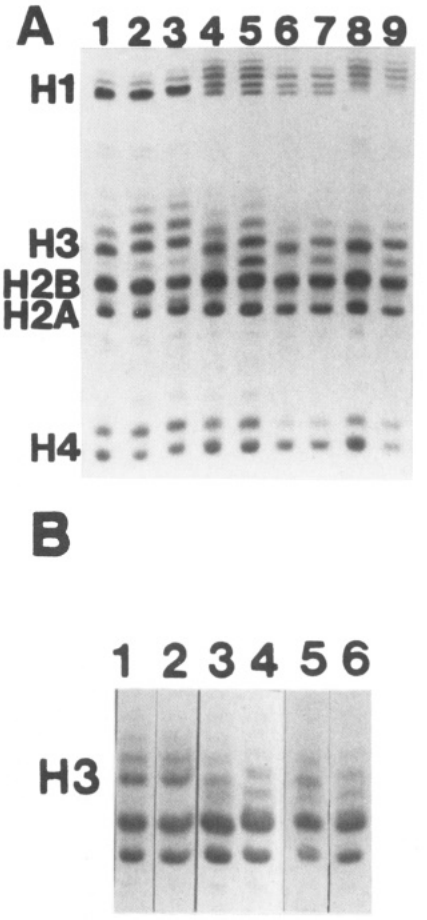

FIGURE 4: Acid–urea gels of histones from control and CMBS-treated samples. (A) Nuclei were purified from rat liver (lanes 1–3), RPMI (lanes 4 and 5), Wilm's tumor (lanes 6 and 7), or breast tumor (lanes 8 and 9) cells in the presence (lanes 2, 3, 5, 7, and 9) or absence (lanes 1, 4, 6, and 8) of 1 mM CMBS. Histones were acid-extracted from the nuclear samples, electrophoresed on a long 32-cm acid–urea/polyacrylamide gel, and stained with Coomassie blue. Histones from CMBS-treated rat liver nuclei were phosphatased and run in lane 3. (B) Nuclei from RPMI cells were purified without CMBS in the absence (lanes 1 and 2 and 5 and 6) or presence (lanes 3 and 4) of IAA. Histones were acid-extracted from control samples and divided into four aliquots: histones in lane 5 received no further treatment; histones in lane 6 were treated with 1 mM CMBS; histones in lane 1 were treated with 10 mM IAA; histones in lane 2 were treated with 10 mM IAA and then with 1 mM CMBS. Histones from nuclei isolated in the presence of 10 mM IAA were divided into two aliquots: histones in lane 3 have no further treatment while histones in lane 4 were treated with CMBS.

In one further experiment, nuclei were isolated in the presence of IAA and the histones isolated (lanes 3 and 4 of Figure 4B). The histones were purified from IAA-exposed nuclei and were incubated without (lane 3) or with (lane 4) CMBS. In this case, CMBS can induce a shift, indicating that IAA cannot bind H3 thiols in nuclei as it can after nuclear histones are purified. Thus, IAA acts similar to *N*-ethylmaleimide, which can react with H3 thiols in mixtures of purified histone but not in intact nuclei (Wong & Candido, 1978). In contrast, the above data indicate that CMBS readily reacts with H3 thiols in nuclei as well as in isolated histones.

H2A Phosphorylation Is Preserved in CMBS-Treated Nuclei. While it is clear from the above results that CMBS covalently attaches to histone H3 thiol groups, it does not follow that the CMBS-induced enhancement in DNase I

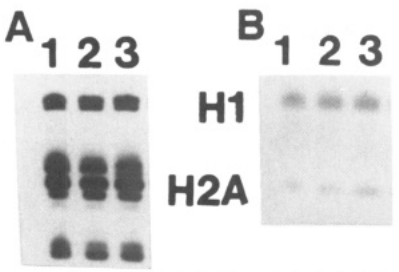

FIGURE 5: Incorporation of labeled phosphate into H1 and H2A in the presence or absence of CMBS during nuclear isolation. RPMI cells were cultured for 3 h in media containing 3 μCi/mL ^{32}P-labeled inorganic phosphate. The cells were harvested, and nuclei were purified by using no inhibitor, 1 mM 5,5′-dithiobis(2-nitrobenzoic acid), or 1 mM CMBS (lanes 1–3, respectively). (A) The histones were acid-extracted from the three samples, electrophoresed on an acid–urea gel, and stained with Coomassie blue. (B) The gel was autoradiographed to display the labeled histones.

sensitivity of nuclei is the result of this particular modification. Histones H1 and H2A are phosphorylated in interphase cells [reviewed in van Holde (1988)], and phosphorylation may enhance DNase I sensitivity by adding negative charges to the histones. Since CMBS is a powerful inhibitor of histone phosphatases (Paulson, 1980), CMBS may enhance the DNase I sensitivity of those chromatin domains containing phosphorylated histones.

To assay for the ability of CMBS to preserve histone phosphorylation in nuclei, RPMI cells were incubated in medium containing ^{32}P-labeled inorganic phosphate (Figure 5). The cells were split into three aliquots, and nuclei were purified by using no inhibitors (lane 1), 5,5′-dithiobis(2-nitrobenzoic acid) (lane 2), or CMBS (lane 3). The nuclei were incubated at 37 °C for 4 min before histone extraction. Figure 5A shows the Coomassie-stained, acid–urea gel containing the purfied histones from the three samples. The autoradiograph of this gel (Figure 5B) shows the level of phosphorylation in H1 and H2A histones with each treatment. While histone H1 phosphorylation appears constant in all three samples, H2A phosphorylation is partially depleted in control nuclei without phosphatase inhibitor (lane 1). CMBS-treated nuclei had the highest H2A phosphorylation (lane 3) while 5,5′-dithiobis(2-nitrobenzoic acid)-treated nuclei (lane 2) had an intermediate level of phosphorylation. Densitometry scans of the autoradiographed samples indicate that the ratio of phosphorylated H2A to phosphorylated H1 in CMBS-treated nuclei is more than 2-fold higher than in control nuclei. Since H2A phosphorylation has been reported to be associated with DNase I sensitive active chromatin (Prentice et al., 1982), these results are consistent with a role for H2A phosphorylation in the CMBS-induced enhancement of active gene DNase I sensitivity.

Discussion

CMBS Enhances the Preferential DNase I Sensitivity of Nuclei with Low Sensitivity. In some cell nuclei, domain DNase I sensitivity can be difficult to detect without CMBS pretreatment (Figure 2 and unpublished data). While inactive domains in RPMI cells have 3-fold higher DNase I sensitivity following treatment with CMBS, DNase I sensitivity increases some 10-fold in active domains. Thus, CMBS strongly enhances the preferential DNase I sensitivity of active regions in RPMI cells. We have found this to be true for HeLa and human HT-1080 cells as well (data not shown), so that CMBS appears to enhance preferential DNase I sensitivity of many transformed cell lines. In contrast, we have detected no enhancement of preferential sensitivity in chick erythrocytes or in normal diploid MRC-5 fibroblasts where DNase I sensitivity is high without CMBS treatment (data not shown). These data suggest that CMBS enhances preferential DNase I sensitivity in nuclei from cells with low preferential DNase I sensitivity whereas little or no effect is seen in nuclei from cells with high preferential DNase I sensitivity.

Possible Mechanisms of CMBS-Enhanced DNase I Sensitivity. Histones are known to bind DNA through electrostatic interactions. Since CMBS is negatively charged, the covalent attachment of CMBS to the histones would tend to loosen their binding to DNA and would likely increase the accessibility of DNA to DNase I. Thus, our observation of CMBS attachment to H3 histone may explain the increases in DNase I sensitivity of treated nuclei.

However, since all or nearly all H3 is modified by CMBS under our treatment conditions, we feel that H3 modification in itself is not sufficient to explain the preferential effects of CMBS on enhancing the DNase I sensitivity of active domains. In support of this interpretation, we found that DNase I sensitivity of active and inactive domains is equally stimulated by DEPC treatment (Figure 3), which indiscriminately modifies nuclear histones (including H3, our unpublished data) by removing positive charge. Moreover, CMBS modifies H3 and increases DNase I sensitivity in bulk chromatin of chick erythrocytes and MRC-5 fibroblasts, yet preferential DNase I sensitivity is not enhanced in these cells (data not shown). These results indicate that while a general loosening of H3 binding to DNA may explain the 3-fold increase in bulk chromatin digestion, it is unlikely to enhance the preferential DNase I sensitivity of active domains.

An alternative to direct CMBS effects on H3 is the possibility that CMBS acts indirectly to increase the preferential DNase I sensitivity of active gene domains by inhibiting degradative enzymes that disrupt chromatin structure. CMBS is a powerful inhibitor of endogenous phosphatases, nucleases, and proteases (Paulson, 1980; our unpublished data) and thus could preserve active chromatin structure in cells with high levels of degradative enzymes.

As an example, we find that the level of H2A phosphorylation is increased in treated nuclei (Figure 5), suggesting that H2A phosphorylation may play a role in maintaining active chromatin structure and preferential sensitivity. Many lines of evidence support the association of H2A phosphorylation with active DNase I sensitive chromatin. First, some 20% of H2A molecules are constitutively phosphorylated (Ruiz-Carrillo et al., 1976; Prentice et al., 1982), which is consistent with the estimated proportion of active chromatin (Weintraub & Groudine, 1976). Second, H2A is highly phosphorylated in the transcriptionally active macronuclei of *Tetrahymena*, whereas H2A is completely unphosphorylated in the inactive heterochromatic micronuclei (Allis & Gorovsky, 1981). Third, a decline in H2A phosphorylation parallels the formation of condensed heterochromatin and the decrease in transcription as duck erythroid cells mature (Ruiz-Carrillo et al., 1976). Fourth, steroid hormones stimulate transcription and increase the level of H2A but not H1 phosphorylation in mouse L cells (Prentice et al., 1978). Fifth, the DNase I sensitivity of chromatin is enhanced some 3–4-fold when H2A becomes hyperphosphorylated (West et al., 1985). Finally, phosphorylated histone H2A is enriched some 3-fold in the DNase I sensitive fraction of mouse L cell chromatin (Prentice et al., 1982). Taken together, our data and these earlier results are consistent with a role for H2A phosphorylation in the maintenance of active DNase I sensitive chromatin. In addition, CMBS inhibits many other degradative enzymes besides

phosphatases so that endogenous nuclease or protease activities may also be responsible for the observed decreased sensitivity of nuclei to DNase I digestion in the absence of CMBS treatment.

Treatment of Nuclei with CMBS and DNase I Provides a Novel Assay for Chromatin Accessibility. While we cannot be certain how CMBS functions, it is clear that CMBS can enhance preferential DNase I sensitivity some 3-fold in cells where DNase I sensitivity is difficult to detect. Moreover, CMBS inhibits phosphatases, nucleases, and proteases in many cell types, so that the nuclei are less degraded and the DNase I assay is more predictable and reliable. These attributes make the combination of CMBS treatment and DNase I sensitivity a novel and reproducible assay for probing the chromatin structure of active domains.

ACKNOWLEDGMENTS

We thank M. Pikaart for helpful criticisms.

Registry No. DNase, 9003-98-9; CMBS, 133128-52-6.

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