# A Chinese Hamster Ovary Cell Histone Deacetylase That Is Associated with a Unique Class of Mononucleosomes†

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ABSTRACT: The chromatin-bound histone deacetylase of Chinese hamster ovary cells has been studied by using as a substrate an acetylated amino-terminal peptide of histone H4. These studies demonstrate that histone deacetylase activity is associated with mononucleosomes solubilized by digestion with micrococcal nuclease. The deacetylase activity remained bound to the nucleosomes, even in the presence of 1 M NaCl. This unique class of deacetylase-associated mononucleosomes is resolved from the major classes of mononucleosomes by polyacrylamide gel electrophoresis. These mononucleosomes contain 290 and 190 base pair DNAs and demonstrate the presence of histone H1 and non-histones HMG-1 and HMG-2 and the absence of HMG-14 and HMG-17. They are further characterized by a specific acetylation pattern of histone H4 and likely represent a functionally important chromatin–DNA complex.

The nucleosome is the fundamental unit of chromatin organization [for reviews, see Igo-Kemenes et al. (1982) and Yaniv and Cereghini (1986)]. Its structure is very heterogeneous due to histone sequence variants, postsynthetic modifications, and the association of non-histone proteins. The heterogeneity is likely to reflect different functional chromatin domains. A well-studied source of nucleosome heterogeneity is that of $N^{\epsilon}$-acetylation of specific lysine residues located near the amino terminal of each of the core histones [for reviews, see Isenberg (1979) and Wu et al. (1986)]. It has been proposed that the reduction of positive charges in the amino-terminal region of the acetylated core histones affects both the sensitivity and accessibility of the nucleosomal DNA to enzymes and regulatory proteins. Indeed, high levels of histone acetylation have been correlated with transcriptionally competent chromatin (Levy-Wilson et al., 1979; Davie & Candido, 1980; Davie et al., 1981; Vavra et al., 1982).

The degree of histone acetylation is regulated by both histone acetyltransferase and histone deacetylase activities. A saline-soluble deacetylase from calf thymus that removes acetyl groups from free histones has been partially purified and characterized (Vidali et al., 1972). Less is known regarding the chromatin-bound form of the enzyme whose substrate is specifically the nucleosomal histones. Recently, Hay and Candido presented evidence that the HeLa cell histone deacetylase is part of a high molecular weight, nuclease-resistant complex (Hay & Candido, 1983a,b). These studies utilized endogenous, acetylated, nucleosomal histones as substrates to demonstrate that the enzyme is bound to the complex through a metalloprotein interaction. This complex does not dissociate from its substrate during the cleavage reaction and is likely to be part of the internal nuclear matrix.

In the studies reported here, a well-defined histone H4[1–23] peptide substrate is used to study the chromatin-bound form of histone deacetylase in Chinese hamster ovary (CHO) cells in order to take advantage of high specific activity and solubility. These studies reveal a histone deacetylase associated class of mononucleosomes likely to function in RNA transcription and/or DNA replication in eukaryotic chromatin. The deacetylase activity identifies both a unique histone deacetylase associated class of mononucleosomes and a specific histone acetylation pattern.

## Materials and Methods

*Cell Culture Conditions.* CHO-K1 cells were grown in monolayer cultures in Eagle's minimal essential medium (MEM) supplemented with nonessential amino acids and vitamins, containing 10% fetal bovine serum, 100 units/mL penicillin, and 30 μg/mL gentamicin.

*Isolation of Nuclei.* Five 150 $cm^2$ flasks of CHO-K1 cells were grown to confluence and washed 4 times at room temperature with 50 mL of calcium- and magnesium-free Dulbecco's phosphate-buffered saline containing 1 mM ethylene glycol bis(β-aminoethyl ether)-*N,N,N′,N′*-tetraacetic acid (EGTA) (CMF–PBS–EGTA). The cells were then detached into 15 mL of CMF–PBS–EGTA by pipetting and collected by centrifugation at 1100*g* for 5 min. They were then chilled to 5 °C and washed twice with 15 mL of CMF–PBS–EGTA and once with 15 mL of 10 mM tris(hydroxymethyl)aminomethane hydrochloride (Tris-HCl), pH 7.4, 10 mM NaCl, and 5 mM $MgCl_2$ (RSB). The cells were then allowed to swell for 10 min in 15 mL of RSB. The swollen cells were collected by centrifugation, resuspended in 5 mL of 10 mM Tris-HCl, pH 7.4, 0.25 M sucrose, 25 mM KCl, 3 mM $MgCl_2$, and 1 mM $CaCl_2$ (NSB) containing 1% Triton X-100, and immediately forced twice through a 22-gauge needle. The homogenate was adjusted to 15 mL with NSB containing 1% Triton X-100, and the nuclei were collected by centrifugation at 1600*g* for 7.5 min. The nuclei were washed once with NSB and resuspended in 1.0 mL of NSB for digestion with micrococcal nuclease.

*Micrococcal Nuclease Digestion.* Micrococcal nuclease was added to the nuclear suspension at a concentration of 2 units/$A_{260}$ unit of DNA (for the average preparation of nuclei, approximately 75–100 units/mL of nuclear suspension), and digestion was carried out for 25 min at 25 °C. Digestion was terminated by the addition of 20 mM EGTA, pH 7.4, to a final concentration of 2 mM. The nuclei were chilled on ice and lysed by overnight dialysis against 2000 volumes of 10 mM Tris-HCl buffer, pH 7.4, containing 1 mM ethylenediaminetetraacetic acid (EDTA).

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*Fractionation of Nucleosomes on Sucrose Gradients.* The nuclear lysate was clarified by centrifugation at 8000*g* for 15 min. Seven-tenths of a milliliter of nuclear lysate was layered onto a 10.75-mL, linear, 5–20% sucrose gradient containing 10 mM Tris-HCl buffer, pH 7.4, and 1 mM EDTA and centrifuged at 35 000 rpm for 13.5 h in a Beckman SW41 rotor. Gradients were unloaded by upward displacement with 40% (w/v), sucrose and the fractions were continuously monitored for the absorbance at 254 nm with an LKB 2138 Uvicord detector.

*Preparation of the Peptide Substrate.* Calf thymus histone H4 was dissolved at a concentration of 10 mg/mL in 0.25 M acetic acid and heated in a sealed tube for 6 h at 105 °C. The solution was cooled, lyophilized, dissolved in 8 M urea–20 mM HCl, and loaded on a column of Sephadex G-50 (125 × 2.5 cm). The peptide fragments were eluted from the column with 20 mM HCl–0.02% sodium azide. Peptide H4[1–23] and peptide H4[69–102] eluted as a single peak. These two peptides were separated by preparative electrophoresis as follows. The lyophilized sample from the G-50 column containing 17.2 mg of peptide was dissolved in 2.0 mL of sample buffer (5% acetic acid, 15% sucrose, and 0.0025% pyronin Y). One milliliter of the sample was loaded across the top of a 30% polyacrylamide/acid–urea gel (12.5 × 13.5 × 0.075 cm). Peptide H4[1–23], which migrates faster than peptide H4-[69–102] under these conditions, was electrophoresed out of the polyacrylamide gel and collected in 130 mL of 5% acetic acid reservoir buffer. The reservoir buffer was lyophilized, and the peptide was resuspended in 5.0 mL of 0.02 N HCl and precipitated with 50 mL of acidified (0.1% HCl) acetone. The precipitate was collected by centrifugation, dried under a stream of nitrogen, and dissolved in distilled water.

The purified peptide H4[1–23] was labeled with [$^3$H]acetate in the following manner. One milligram of the purified peptide was dissolved in 1.0 mL of 25 mM sodium bicarbonate, pH 9.0. Twenty-five millicuries of [$^3$H]acetic anhydride (9.39 Ci/mmol, Amersham) was transferred by vacuum distillation into the H4 peptide solution. Two microliters of 1 N NaOH was added to readjust the pH to 9.0. After a 30-min incubation at room temperature, the mixture was chromatographed over Sephadex G-15 (1.0 × 40.0 cm), which had been equilibrated with 0.5 M acetic acid, in order to desorb any free acetate from the labeled acetylated peptide. The void volume was collected, lyophilized, and dissolved in distilled water at a concentration of 0.046 mg/mL (3.0 × 10 cpm/mL).

*Assay for Histone Deacetylase Activity.* The histone deacetylase assay is as described by Waterborg and Matthews (1982) with the following modifications. Twenty microliters of enzyme was added to 20 μL of 25 mM Tris-HCl buffer, pH 8.0, containing 0.5 μL of tritiated, acetylated peptide H4[1–23]. The mixture was incubated for 1 h at 37 °C, and the reaction was stopped by the addition of 20 μL of a solution containing 5 mg/mL calf thymus histones in 0.5 N HCl, and 0.08 M acetic acid. Three hundred microliters of ethyl acetate was added, and the tubes were vigorously vortexed twice. The two phases were separated by centrifugation, 200 μL of the upper phase was removed and mixed with 5 mL of scintillation cocktail, and the radioactivity was determined. Nonenzymatic release of acetate was determined and subtracted from the total in all assays. The extraction efficiency of liberated acetic acid from the reaction mixture was consistently at 51.8%. A second extraction with ethyl acetate only increased the extraction efficiency to 69.0% and was not routinely carried out.

*One-Dimensional Gel Electrophoresis of Nucleoprotein Complexes.* Nucleoprotein complexes were separated electrophoretically using 4% [acrylamide:*N*,*N*′-methylenebis-(acrylamide) ratio of 25:1] slab gels (12.5 × 13.5 × 0.075 cm) containing 6.4 mM Tris, 3.2 mM sodium acetate, and 0.32 mM EDTA, pH 8.0. Nucleosome-containing fractions from the sucrose density gradients were loaded directly on the gel following their concentration using Amicon Centricon 30 concentrators and the addition of bromphenol blue as a tracking dye. Electrophoresis with recirculating buffer was carried out at 50 V at room temperature until the tracking dye had completely entered the gel. The voltage was then increased to 150 V, and electrophoresis was continued for 45 min after the tracking dye had reached the bottom of the gel.

*Two-Dimensional Gel Electrophoresis To Display Double-Stranded DNA.* The first-dimension gel strips (1.0 × 12.5 × 0.075 cm) were soaked for 1 h at 50 °C in 0.5× TBE buffer (89 mM Tris–borate, 89 mM boric acid, and 2 mM EDTA) containing 1% sodium dodecyl sulfate (SDS) and 0.005% bromphenol blue. The gel slice was laid in the sample well of a 4% Nusieve agarose wick gel (16 × 19 cm) containing 1× TBE buffer and sealed with agarose. Electrophoresis was carried out until the dye front reached the bottom of the gel. The gel was stained with 1 μg/mL ethidium bromide for 1 h.

*Two-Dimensional Gel Electrophoresis To Display Proteins.* Proteins of the electrophoretically separated nucleoprotein complexes were displayed by electrophoresis in a second dimension using Triton–acid–urea slab gels (12.5 × 12.5 × 0.15 cm) and the protamine release method of Richards and Shaw (1982). After electrophoresis of the nucleoprotein in the first dimension, a strip (1.0 × 12.5 × 0.07 cm) was cut from the slabs and soaked in 50 mL of Triton–acid–urea gel sample buffer (8 M urea, 5% β-mercaptoethanol, 2.5% thioglycolic acid, and 5% acetic acid) containing 0.0025% pyronin Y. The strip was then positioned across the top of a second-dimension Triton–acid–urea gel, sealed with 1% agarose, and overlayed with gel sample buffer containing 1% protamine sulfate. Electrophoresis was performed at 150 V for 15 h at 25 °C. Following electrophoresis, the gels were silver stained by the method of Mold et al. (1983).

*Assay of Deacetylase Activity in Gel Slices.* The standard assay for histone deacetylase was modified to allow detection of activity in gel slices. Specifically, the incubation and ethyl acetate extraction periods were lengthened to enable the peptide substrate and acetate reaction product time to diffuse in and out of the gel slice. Each gel slice (0.5 × 1.0 cm) was cut into 16 pieces and placed in the bottom of a 1.5-mL microfuge tube. Sixty microliters of 25 mM Tris-HCl, pH 7.4, containing 2 μL of tritiated, acetylated peptide H4[1–23] was added to each tube, and the mixture was incubated at 37 °C for 4 h. The reaction was stopped by the addition of 30 μL of carrier histone in 0.5 N HCl and 0.08 M acetic acid. Four hundred and fifty microliters of ethyl acetate was added, and the liberated acetate was extracted by vigorously shaking the assay tubes for 1 h. The phases were separated by centrifugation, and 300 μL of the upper phase was removed and added to 5 mL of the scintillation solution and counted. Appropriate corrections were made for the nonenzymatic release of acetate.

## RESULTS

Chinese hamster ovary cell nuclei were digested with micrococcal nuclease at 25 °C. The nuclei were lysed, and insoluble material was removed by centrifugation. Assay of histone deacetylase activity using acetylated peptide H4[1–23] as a substrate revealed that 76.6% of the total nuclear deacetylase activity was located in the soluble chromatin fraction after micrococcal nuclease digestion (Table I). This material

Table I: Distribution of Histone Deacetylase Activity in Nuclear Fractions

| | deacetylase activity (%)[a] |
|---|---|
| nuclear lysate | 100.0 |
| soluble chromatin fraction | 76.6 |
| sucrose gradient fractions | 25.6 |
| 4S gradient peak | 4.6 |
| mononucleosome fraction | 9.7 |

[a] Values represent percent of the total nuclear deacetylase activity and refer to the results of the experiment shown in Figure 1.

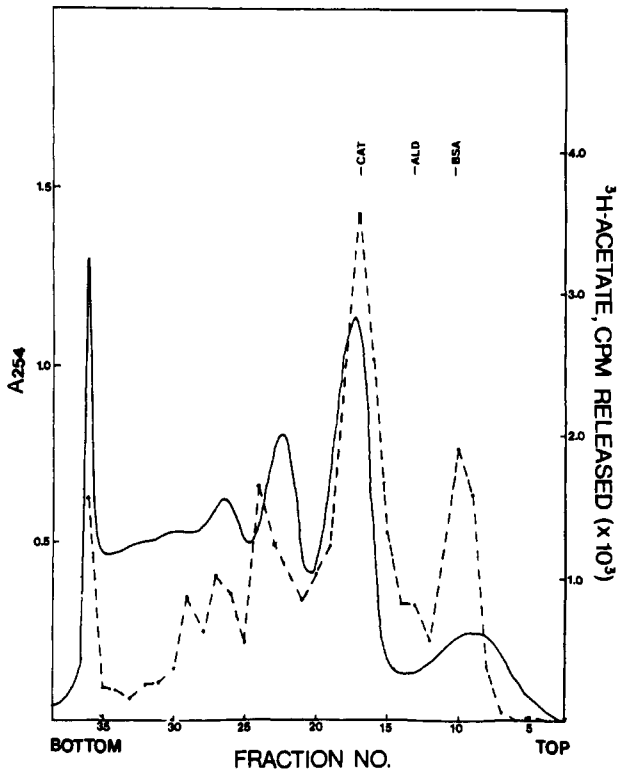


FIGURE 1: Sucrose gradient profile of nucleosomes and histone deacetylase activity. CHO cell nuclei were digested with micrococcal nuclease, and nucleosomes were isolated by sedimentation through a linear, 5–20% sucrose gradient in a Beckman SW41 rotor at 35000 rpm for 13.5 h. (—) $A_{254}$; (---) [$^3$H]acetate, cpm released ($\times 10^3$). Standards sedimented on a parallel gradient are indicated by BSA [bovine serum albumin (4.22 S)], ALD [rabbit muscle aldolase (7.35 S)], and CAT [bovine catalase (11.0 S)].

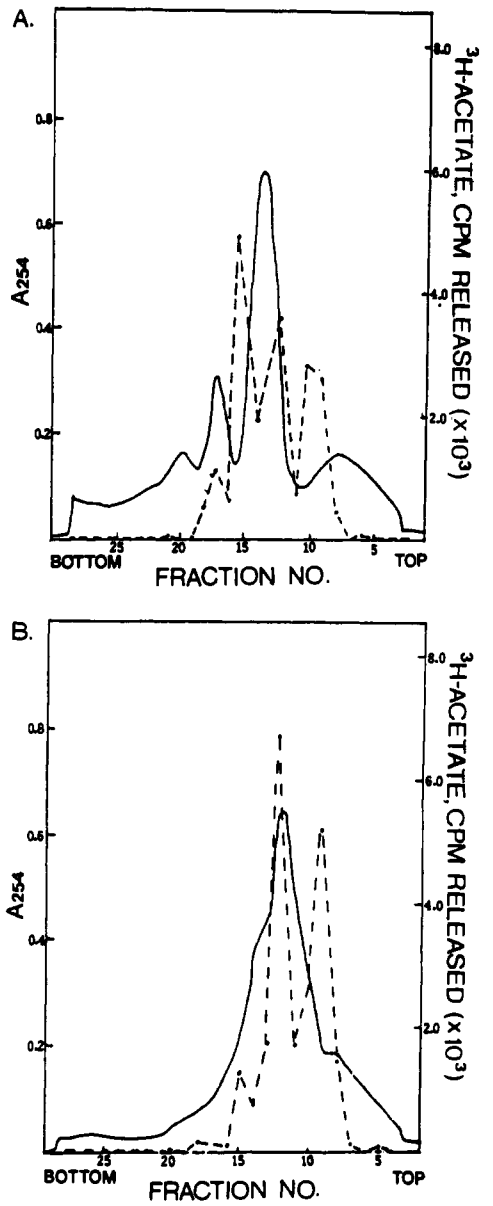


FIGURE 2: Effect of NaCl concentration on the association of histone deacetylase with nucleosomes. Micrococcal digests of CHO cell nuclei were fractionated on sucrose gradients in the presence of (A) 0.5 M NaCl and (B) 1.0 M NaCl. (—) $A_{254}$; (---) [$^3$H]acetate, cpm released ($\times 10^3$).

was further fractionated by sedimentation in a linear sucrose gradient. The gradient fractions were assayed for nucleoprotein by the absorbance at 254 nm and for histone deacetylase activity. A typical sucrose gradient profile is shown in Figure 1. Of the activity that was loaded on the gradient, 33% was recovered in the gradient fractions. This represents 25.6% of the total nuclear deacetylase activity (Table I). The remainder of the activity pelleted to the bottom of the centrifuge tube. The nucleoprotein profile revealed peaks corresponding to monomer, dimer, trimer, and oligomer nucleosomes. A small peak of activity is observed near the top of the gradient. This peak, which sediments at approximately 4 S when compared to a series of sedimentation standards (Figure 1), represents 4.6% of the total nuclear deacetylase activity (Table I) and is presumed to be dissociated enzyme. The remainder of the enzyme activity in the gradient fractions is associated with the nucleosome peaks. In the experiment shown in Figure 1, 9.7% of the total nuclear deacetylase activity is associated with the 11S mononucleosome peak. The proportion of activity distributed among mono-, di-, and trinucleosomes varied slightly with each preparation of nuclei (see Figure 2A), due to subtle differences in the extent of micrococcal nuclease digestion.

It is important to exclude any likelihood of nonspecific adsorption of the deacetylase activity. For this, fractions from each of the nucleosome peaks were pooled, dialyzed to remove the sucrose, concentrated, and then reapplied to a new sucrose gradient. In each case, all of the histone deacetylase activity appeared to be associated with the nucleoprotein peaks. The effect of salt concentration on the association of histone deacetylase with nucleosomes was then examined. For these studies, the nuclease digests were fractionated on sucrose gradients in the presence of 0.5 M NaCl and 1.0 M NaCl. In the presence of 0.5 M NaCl (Figure 2A), the majority of the histone deacetylase activity sedimented with the nucleosomes. As anticipated, the nucleosomes sediment more slowly under these conditions due to the dissociation and loss of most of the histone H1 and other non-histone chromosomal proteins (Gaubatz & Chalkley, 1977). The sedimentation of the histone deacetylase associated class of nucleosomes was more affected by the removal of these proteins than the major classes

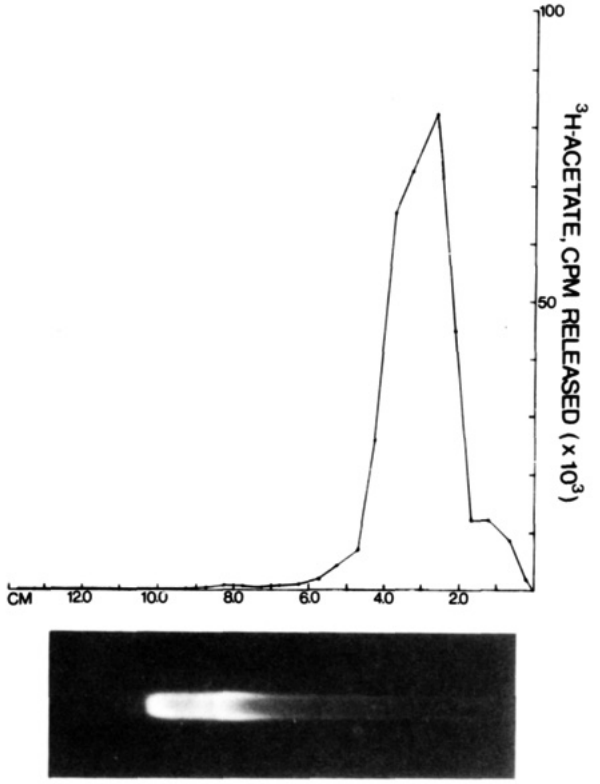


FIGURE 3: Histone deacetylase activity associated with mononucleosomes fractionated by polyacrylamide gel electrophoresis. Mononucleosome-containing fractions from sucrose density gradients were pooled, concentrated, and electrophoresed in low ionic strength, 4% polyacrylamide gels. Nucleoprotein was detected by staining with ethidium bromide. Histone deacetylase activity was measured in gel slices as described under Materials and Methods and is plotted as a function of distance in centimeters from the top of the gel. (—) [$^3$H]Acetate, cpm released (×10$^3$).

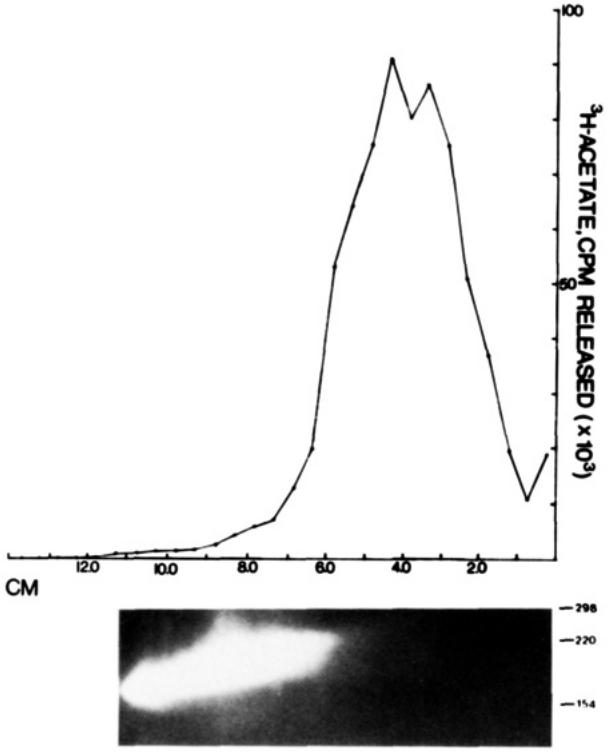


FIGURE 4: Second-dimension DNA mapping of electrophoretically separated mononucleosomes. Mononucleosomes isolated from sucrose density gradients were separated by electrophoresis in a low ionic strength, 4% polyacrylamide gel in the first dimension. After the first-dimension gel slice was treated with 1.0% SDS (see Materials and Methods), the DNA was displayed by performing electrophoresis in a second dimension using 4% Nusieve agarose. The histone deacetylase activity profile of the first dimension as determined by assay of gel slices is shown graphically.

of nucleosomes. When an identical sample of micrococcal digest was sedimented in the presence of 1.0 M NaCl, the normal monomer, dimer, trimer, oligomer pattern was no longer detected (Figure 2B). Under these conditions, most of the nucleoprotein is found in a single, broad peak, sedimenting more slowly than mononucleosomes, consistent with the removal of histones H2A and H2B and the sliding and transfer of nucleosome cores. Even at this ionic strength, however, the majority of deacetylase activity sediments as a single peak associated with the nucleoprotein. Only 15% of the total deacetylase activity was observed to dissociate at 1.0 M NaCl, suggesting that the deacetylase is a tightly bound, specific complex.

The enzyme's association with nucleosomes was examined further by fractionating the mononucleosomes by electrophoresis in low percentage, low ionic strength polyacrylamide gels, a technique used to demonstrate nucleosomal heterogeneity (Albright et al., 1980). The deacetylase-associated mononucleosomes separate from the major classes of mononucleosomes, migrating much more slowly in the gel (Figure 3). This fraction represents only a very small percentage of the ethidium-staining DNA, and the enzyme activity was restricted to a sharply defined region of the gel in the absence of a distinct ethidium bromide DNA band. Similar results have been obtained for dinucleosomes (data not shown).

A second-dimension gel electrophoresis was then used to resolve the DNA associated with the deacetylase-containing mononucleosomes. The mononucleosome DNA profile is shown in Figure 4 and is similar to those previously published (Albright et al., 1980; Levinger et al., 1981). In the region of the gel containing deacetylase activity, two ethidium-staining areas are detected, one at about 290 base pairs (bp) and the other at about 190 bp. These results confirm that the deacetylase-associated mononucleosomes contain DNA representative of the mononucleosome size range.

The proteins associated with the different mononucleosome classes of CHO cells were then displayed by electrophoresis in a second-dimension Triton–acid–urea/polyacrylamide gel (Figure 5). Identification of proteins was made by comparing the protein banding patterns with published results (Albright et al., 1980; Zweidler, 1979). Of the two major bands that were resolved in the first dimension, the fastest migrating band was limited to the four core histones and their variant forms and clearly represents the nucleosome core particle. The mononucleosomes of the second major class contain, in addition to the core histones, the presence of the non-histone proteins HMG-1, HMG-2, HMG-14, and HMG-17, histone H1, the minor histone NF, and various unidentified non-histone proteins. The mononucleosomes found in the region of the gel containing the histone deacetylase are distinguished from the major classes of mononucleosomes in the protein pattern. Although the deacetylase-associated mononucleosomes were found to contain histone H1 and the non-histones HMG-1 and HMG-2, they lacked HMG-14 and HMG-17 and the minor histone NF. In addition, the deacetylase-associated mononucleosomes display a different set of unidentified non-histone proteins.

In view of the reports that HMG-14 and HMG-17 inhibit histone deacetylase activity in vitro (Reeves & Candido, 1980), it remains to be determined if the lack of detectable activity in the second major class of mononucleosomes is due to the presence of these proteins. The histone deacetylase activity

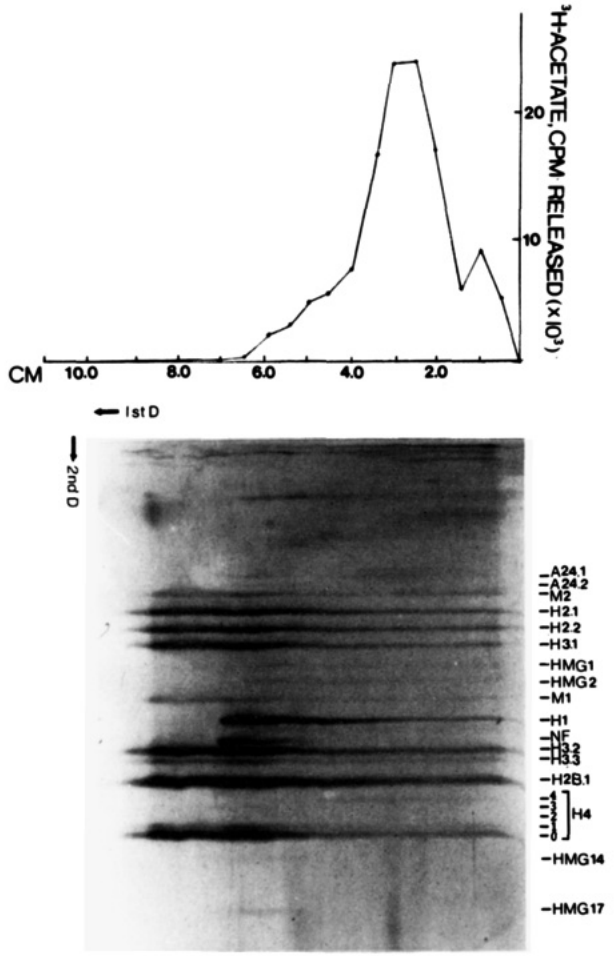


FIGURE 5: Distribution of protein species among different electrophoretic forms of mononucleosomes. Mononucleosomes isolated from sucrose gradients were separated by electrophoresis in a low ionic strength, 4% polyacrylamide gel in the first dimension. A deacetylase activity profile of the first dimension is represented graphically. Proteins were displayed by performing electrophoresis in a second dimension using Triton–acid–urea gels as described under Materials and Methods. The different proteins were identified according to their characteristic migration in this gel system. Protein bands labeled M1, M2, and NF are minor histones. Acetylated forms of histone H4 are bracketed and designated by the following numbers: 0, unacetylated; 1, monoacetylated; 2, diacetylated; 3, triacetylated; 4, tetraacetylated.

of nucleosomes isolated on sucrose gradients run in 0.4 M NaCl was measured, and although a slight increase in the total activity of the mononucleosome population was detected, it does not appear to be sufficient to warrant this conclusion.

The deacetylase-associated mononucleosomes are also distinguished from the other classes by the acetylation pattern of histone H4. The acetylated forms of the core histones exhibit characteristic patterns of migration in Triton–acid–urea gels. The core particles contained a small amount of a band migrating with the mobility of monoacetylated histone H4. The other major class of mononucleosome contained an additional band migrating with the mobility of triacetylated histone H4. In contrast, the histone deacetylase associated mononucleosomes are characterized by the presence of the tetraacetylated form of histone H4 (Figure 5).

## Discussion

These studies demonstrate that histone deacetylase activity is associated with nucleosomes released from Chinese hamster ovary cell nuclei following micrococcal nuclease treatment. As previously suggested (Levy-Wilson, 1979), this could be the consequence of a rearrangement of chromosomal proteins during the nuclease digestion. There are several strong arguments, however, in favor of this being a specific interaction. First, the histone deacetylase is tightly bound to the nucleosomes and does not dissociate from the nucleosomes on sucrose gradients in the presence of 0.5 M NaCl. Second, the enzyme is specifically associated with a special class of mononucleosomes. Rearrangements and aggregation of proteins would be expected to result in a more random distribution of deacetylase activity among nucleosomes.

Hay and Candido, using in vivo acetylated histones, did not detect any histone deacetylase activity associated with mononucleosomes in their study of the chromatin-bound histone deacetylase of HeLa cells (Hay & Candido, 1983a,b). Instead, they found enzyme activity only in association with a micrococcal nuclease resistant, high molecular weight, chromatin complex. One explanation for this discrepancy is that the assay for histone deacetylase activity used in our study is more sensitive due to the use of a chemically acetylated peptide substrate. Peptide H4[1–23] contains only four lysines (residues 5, 8, 12, and 16), all of which may be acetylated and deacetylated in H4 in vivo (DeLange et al., 1969; Dixon et al., 1975), and we were able to specifically label them in vitro with acetic anhydride to a very high specific activity. Histones that are labeled with acetate in vivo give substrates with a relatively low specific activity, resulting in assays that are 100–1000 times less sensitive than those that utilize the chemically acetylated H4[1–23] as a substrate (Waterborg & Matthews, 1982).

An alternative explanation for the discrepancy in results has as its basis the methods used to effect lysis of the nuclei following micrococcal nuclease digestion. Hay and Candido, in their experiments, used a small volume of a hypotonic solution containing a minimum concentration of EDTA to lyse the HeLa cell nuclei. In contrast, nuclear lysis was accomplished in our experiments by dialysis against a very large volume of a hypotonic solution containing a much higher concentration of EDTA. It has been demonstrated that if care is taken to prevent removal of $Mg^{2+}$ during the isolation of nuclei and digestion with micrococcal nuclease, the differential packing of native chromatin is left undisturbed (Sanders, 1978). If, in our experiments, enough $Mg^{2+}$ was removed during the lysis of nuclei to destabilize the native chromatin packing, some nuclease-resistant mononucleosomes, which usually remain insoluble at low ionic strength, may have been released. It should be noted that although we detected activity in the mononucleosome fraction, we found the majority of the activity to be associated with high molecular weight material that pelleted to the bottom of the sucrose gradients (Table I).

The deacetylase-associated mononucleosomes migrate much more slowly in nondenaturing polyacrylamide gels than the majority of the mononucleosomes. In fact, their mobility is less than that of the major dinucleosome class. Since this unique class of mononucleosomes sediments with other mononucleosomes on sucrose gradients, suggesting a similar size, the proteins associated with them must impart a significant charge difference to account for the difference in their electrophoretic mobility.

Two size classes of DNA were found in the deacetylase-associated mononucleosomes. Although this could be the consequence of a nonspecific aggregation, it appears to be unlikely since the deacetylase-associated mononucleosomes sediment as monomers on sucrose gradients. It is also possible that these represent DNAs from two separate classes of mononucleosomes comigrating under these conditions. We propose that the deacetylase enzyme complex binds to the linker

DNA. The two sizes of DNA may represent mononucleosomes with enzyme bound to linker DNA restricted either to one or to both sides of the nucleosome.

An important characteristic that distinguishes the deacetylase-associated mononucleosomes from other classes of mononucleosomes is their enrichment in tetraacetylated histone H4. One could speculate that different acetylated forms of histone H4 are required for different chromatin functions and that the proportions of these are regulated by deacetylases of different specificites. On the basis of studies of histone acetylation patterns in Physarum (Matthews & Bradbury, 1982) and sea urchins (Chambers & Shaw, 1984), it appears that the diacetylated form of histone H4 may be necessary to induce the changes in chromatin structure that precede replication and that tetraacetylation of H4 may be a prerequisite for RNA transcription.

It has recently been established that there are specific attachment sites for proteins of the nuclear matrix that are spaced along the DNA (Mirkovitch et al., 1984; Gasser & Laemmli, 1986). These attachment sites appear to be critical to the organization of the chromatin into looped, functional domains (Cook & Brazell, 1975; Benyajati & Worcel, 1976). It has also been suggested that the chromatin-bound histone deacetylase of HeLa cells is part of the nuclear matrix. The histone deacetylase associated mononucleosomes reported here may represent a unique class of nucleosomes that may contain the DNA that serves as matrix attachment sites. During the lysis of nuclei and subsequent release of nucleosomes, in the experiments described here, it is possible that the interior nuclear matrix was disrupted and that fragments of the matrix containing the histone deacetylase remained bound to the DNA at their matrix attachment sites. If this is true, then our fractionation technique may be a valued technique for the isolation and characterization of this functionally important DNA.

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