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Histone Deacetylase from HeLa Cells: Properties of the High Molecular Weight Complex†

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ABSTRACT: In previous work [Hay, C. W., & Candido, E. P. M. (1983) *J. Biol. Chem. 258*, 3726–3734], we have shown that the histone deacetylase from HeLa cell nuclei is associated with a high molecular weight, nuclease-resistant complex. This complex was found to contain a variety of non-histone proteins, and indirect evidence for the importance of protein–protein interactions in the maintenance of its structure was obtained. In the present report, we examine the effects of β-mercaptoethanol and neocuproine on the deacetylase complex and present data on the level of histone acetylation and the presence of satellite DNA sequences in this material. HeLa cell histone deacetylase complex partially dissociates in 10 mM β-mercaptoethanol, resulting in a loss of non-histone proteins. The presence of 10 mM β-mercaptoethanol during the micrococcal nuclease digestion of HeLa cell nuclei results in a greatly reduced yield of histone deacetylase complex, with a correspondingly large increase in the production of small oligonucleosomes and mononucleosomes. Histone deacetylase activity on endogenous labeled histone within the complex is strongly inhibited by either 1 or 10 mM β-mercaptoethanol or 3 mM neocuproine. This loss of histone deacetylase activity does not seem to be due to an inactivation of the enzyme but appears to be a consequence of the disruption of the structure of the deacetylase complex itself. Histone H4 in the deacetylase complex prepared from HeLa cell nuclei by micrococcal nuclease digestion was more highly acetylated than H4 in bulk nucleosomes. Restriction enzyme analysis of the DNA associated with the histone deacetylase complex revealed neither an enrichment nor a depletion of major satellite sequences in this material.

Nucleosomes, the basic unit of chromatin organization, have been well characterized structurally (McGhee & Felsenfeld, 1980). Although the amino acid sequence of the nucleosome core histones H2A, H2B, H3, and H4 are highly conserved, the histones exhibit considerable heterogeneity which is created by a variety of posttranslational modifications including acetylation, methylation, phosphorylation, and ADP-ribosylation (Elgin & Weintraub, 1975; Ogata et al., 1980). As these modifications can cause considerable alterations in the charge distributions of histones and are incomplete in nature, they introduce heterogeneity into the chromatin structure. One of the best-studied modifications is the reversible acetylation at ε-amino groups of specific lysyl residues located near the amino terminal of each core histone (Dixon et al., 1975).

While the exact function of histone acetylation remains unclear, it has been proposed that the reduction in the positive charge in the acetylated core histones may alter the conformation of chromatin and result in a concomitant increase in the accessibility of the DNA to specific nuclear enzymes or regulatory proteins. This suggestion is based upon correlations between high levels of gene activity and high levels of histone acetylation (Vavra et al., 1982; Davie et al., 1981) and on the observation that the nucleosomes of transcriptionally competent chromatin have high levels of histone acetylation (Davie & Candido, 1980; Levy-Wilson et al., 1979). Recently, it has also been suggested that all of the chromatin in a cell may experience cycles of acetylation and deacetylation which may

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open up the chromatin for inspection and repair (Perry & Chalkley, 1982).

In spite of recent advances in our knowledge of chromatin structure, uncertainty surrounds the higher order organization of nucleosomes. There is accumulating evidence that chromatin takes the form of loops of 50–100 kilobase pairs (kbp) which are anchored to a protein framework (Adolph, 1980; Adolph et al., 1977; Laemmli et al., 1978). These matrix structures have been studied in several different cell lines and animal tissues. Detke & Keller (1982) have described a nucleoskeleton in HeLa cells which has DNA and protein as the major structural elements and is found during all stages of the cell cycle except mitosis. On the other hand, the chromosome scaffold present during mitosis contains many of the proteins found in the nucleoskeleton. Berezney & Buchholtz (1981) have also isolated large protein and DNA matrices from rat liver nuclei after treatment with nuclease and high concentrations of NaCl. Further evidence that chromatin fibers are constrained by a protein framework comes from the recent findings of Lewis & Laemmli (1982) that high molecular weight protein scaffolds can be derived from metaphase chromosomes upon extraction of the histones.

Our recent finding that histone deacetylase is associated with a high molecular weight, nuclease-resistant complex (Hay & Candido, 1983) led us to investigate the characteristics of the histone deacetylase complex. This in vivo assembled system should assist investigations into the function of histone acetylation and perhaps increase our understanding of the protein matrix within nuclei.

Experimental Procedures

Preparation of Histone Deacetylase Complex. Histone deacetylase complex was prepared from HeLa nuclei by partial micrococcal nuclease digestion as described previously (Hay & Candido, 1983). Briefly, frozen (−80 °C) acetate-labeled cell pellets were homogenized gently in 4 volumes of TMKS [0.05 M tris(hydroxymethyl)aminomethane hydrochloride (Tris-HCl), pH 7.4, 3 mM $MgCl_2$, 25 mM KCl, 0.25 M sucrose, 40 mM sodium butyrate, and 0.1 mM phenylmethanesulfonyl fluoride (PMSF)] and centrifuged at 3000*g* for 7 min. The pellet was rehomogenized and centrifuged twice as before. The resulting pellet was suspended in TMKS made 1 mM in $CaCl_2$, micrococcal nuclease (Sigma) was added at 2.5 A_{260} units of enzyme/A_{260} of nuclei, and digestion was carried out for 5 min at 25 °C. Ethylene glycol bis(β-aminoethyl ether)-*N*,*N*,*N*′,*N*′-tetraacetic acid (EGTA) was then added to a final concentration of 3 mM, the chilled nuclear suspension was centrifuged at 5000*g* for 10 min, and the supernatant was collected. The nuclei were lysed for 30 min on ice with buffer A [10 mM Tris-HCl, pH 7.4, 0.2 mM ethylenediaminetetraacetic acid (EDTA), 50 mM sodium butyrate, and 0.1 mM PMSF] and centrifuged at 12000*g* for 20 min, and this supernatant was combined with the first. The combined supernatants were applied to a Bio-Gel A-50m column (1.5 × 90 cm) at 4 °C. The column buffer contained 10 mM Tris-HCl, pH 7.4, 0.7 mM EDTA, and 50 mM sodium butyrate unless stated otherwise. Appropriate fractions containing histone deacetylase complex were collected and pooled.

Histone Deacetylase Assays. Samples of [^{3}H]acetate-labeled histone deacetylase complex which were used to investigate the effects of neocuproine were dialyzed for 2 h against buffer A (10 mM Tris-HCl, pH 7.4, 0.7 mM EDTA, and 50 mM sodium butyrate) which either was unmodified or contained 3 mM neocuproine. For experiments on the effect of mercaptoethanol, equal aliquots of acetate-labeled histone deacetylase complex were either untreated, boiled for 1 min, or made 1 or 10 mM in β-mercaptoethanol. Sodium butyrate was removed from all samples of histone deacetylase complex by dialysis for 3 h at 4 °C against buffer B (10 mM Tris-HCl, pH 7.8, and 20 mM NaCl) or against buffer B containing the same concentration of reagent as the sample being dialyzed.

Histone deacetylase activity in histone deacetylase complex and nuclear matrix preparations was measured by incubating the samples for 2 h at 22 °C and terminating the reaction by boiling for 1 min. Released [^{3}H]acetate was extracted and counted as described (Hay & Candido, 1983).

Precipitation of Nucleosomes and Histone Deacetylase Complexes. Appropriate Bio-Gel A-50m column fractions containing histone deacetylase complexes or nucleosomes were pooled and concentrated under nitrogen by using an Amicon concentrator fitted with a YM10 membrane. The concentrate was made 0.2 M in sodium acetate, and 50 μg of ultrapure bovine serum albumin (Calbiochem) was added as carrier. The samples were precipitated overnight at −20 °C upon the addition of 6 volumes of −20 °C ethanol. The precipitates were collected by centrifugation at 16000*g* for 15 min, and the pellets were washed in −20 °C ethanol and collected by centrifugation as before. The pellets were dried in vacuo for 10 min at room temperature and used for sodium dodecyl sulfate (SDS)–polyacrylamide gel electrophoresis, acid–urea gel electrophoresis, or DNA preparation.

Agarose Gel Electrophoresis. Electrophoresis of DNA was carried out on horizontal slab gels of 0.75% agarose in 89 mM Tris–boric acid (pH 8.3), 2.5 mM EDTA, and 5 μg/mL ethidium bromide, and the DNA bands were visualized under UV light.

Acid–Urea Gel Electrophoresis. Polyacrylamide acetic acid–urea slab gels (0.08 cm × 7.5 cm × 10 cm) were made according to the methods of Davie (1982). The resolving gel contained 15% acrylamide in 2.5 M urea–5.4% acetic acid, and preelectrophoresis was carried out for 2 h at 200 V, 4 °C. The stacking gel was 5% acrylamide in 2.5 M urea and 0.375 M potassium acetate (pH 4.0). The gel was run at 100 V for 7 h at 4 °C and stained with 0.25% Coomassie Blue.

Restriction Enzyme Digestions. Restriction enzyme digestions of DNA were carried out according to the manufacturer's (BRL) instructions.

Results and Discussion

Effect of β-Mercaptoethanol and Neocuproine on the Histone Deacetylase Complex. Since copper chelation with 3 mM neocuproine or treatment with β-mercaptoethanol causes the dissociation of histone-depleted chromosome scaffolds (Lewis & Laemmli, 1982), the effect of these reagents on the histone deacetylase complex was investigated. [^{3}H]Acetate-labeled histone deacetylase complex was prepared from labeled HeLa cell nuclei in the presence of 50 mM sodium butyrate as previously described (Hay & Candido, 1983). The pooled fractions of histone deacetylase complex were divided into equal aliquots, treated as outlined in Table I, and assayed for histone deacetylase activity upon removal of sodium butyrate by dialysis. A parallel set of untreated samples served as a control to determine the normal level of enzyme activity. Each assay released 208 000 cpm, representing 48% of the total present. The presence of β-mercaptoethanol at concentrations of either 1 or 10 mM caused a strong inhibition of the endogenous histone deacetylase in the complex. In order to establish whether this inhibition was reversible, samples of labeled histone deacetylase complex were made 10 mM in β-mercaptoethanol as before, but β-mercaptoethanol was omitted during the dialysis used to remove sodium butyrate. In this manner, the histone deacetylase complex was exposed

Table I: Effect of β-Mercaptoethanol and Neocuproine on Histone Deacetylase Complex[a]

treatment of histone deacetylase complex prior to dialysis to remove butyrate	treatment of histone deacetylase complex during dialysis to remove butyrate	[^{3}H]acetate released (%) (less background)
none	none	48
1 mM β-mercaptoethanol	1 mM β-mercaptoethanol	14
10 mM β-mercaptoethanol	10 mM β-mercaptoethanol	14
10 mM β-mercaptoethanol	none	18
dialyzed for 2 h against buffer A containing 3 mM neocuproine	3 mM neocuproine	15
dialyzed for 2 h against buffer A	none	40

[a] Equal samples of [^{3}H]acetate-labeled histone deacetylase complex were dialyzed against buffer B (10 mM Tris-HCl, pH 7.8, and 20 mM NaCl) to remove butyrate after either being modified or being dialyzed against buffer A (10 mM Tris-HCl, pH 7.4, 0.7 mM EDTA, and 50 mM sodium butyrate). The assays were performed in triplicate each time. The background release of [^{3}H]acetate from labeled histone deacetylase complex was measured by boiling samples for 1 min prior to dialysis to remove butyrate and assaying for histone deacetylase. This background was found to be 9% of the total counts present.

to 10 mM β-mercaptoethanol, but none was present during the enzyme assay. Although the enzyme activity was slightly higher than in the previous case where β-mercaptoethanol was present during the assay, the activity remained substantially lower than in the untreated control assays, only 18% of the total counts being released. Therefore, it appears from these results that the inhibition of histone deacetylase by β-mercaptoethanol is irreversible.

Neocuproine is a strong copper ion chelator (Nebesar, 1964), and in order to observe its effect on histone deacetylase activity, samples of the [^{3}H]acetate-labeled complex were dialyzed against buffer A containing 3 mM neocuproine for 2 h at 4 °C and assayed. As controls, equal aliquots of the complex were treated in the same manner but without the addition of neocuproine. The results in Table I clearly show that exposure of the histone deacetylase complex to 3 mM neocuproine causes a strong inhibition of histone deacetylase activity, approximately equal to that caused by β-mercaptoethanol.

The inhibition by β-mercaptoethanol could have occurred as a result of inactivation of the enzyme or by disruption of the histone deacetylase complex. The effect of β-mercaptoethanol on enzyme activity was examined further by preparing histone deacetylase complex from unlabeled HeLa cell nuclei and using free acetate labeled histones as the substrate. Aliquots of unlabeled histone deacetylase complex were incubated with free labeled histone either with or without 10 mM β-mercaptoethanol, as shown in Table II. Parallel samples of histone deacetylase complex were boiled prior to incubation with the labeled histone to measure the background release of radioactivity. Although only 8% of the total counts present in the added histones were released, there was no difference in the histone deacetylase activity between assays containing β-mercaptoethanol and those free of the reducing agent. The β-mercaptoethanol at these concentrations thus seems to affect the interaction between histone deacetylase and chromatin, rather than the enzyme itself.

Advantage was taken of the very high molecular weight of the histone deacetylase complex to perform a series of simple experiments examining the possibility that β-mercaptoethanol might disrupt the structure of this complex. In the first experiment, a micrococcal nuclease digest of HeLa cell nuclei was applied to a Bio-Gel A-50m column, and the excluded peak (histone deacetylase complex) was collected and reapplied to the same column. All of the material which absorbed at 260 nm was recovered in the excluded peak as shown in Figure 1A. Thus, reisolation of the histone deacetylase complex by a second passage through a Bio-Gel A-50m column did not cause dissociation. However, when the experiment was repeated in the presence of 10 mM β-mercaptoethanol in the

Table II: Effect of β-Mercaptoethanol on Histone Deacetylase[a]

β-mercaptoethanol (mM) added to assays	[^{3}H]acetate released (cpm)
0	2171
1	2201
10	2145

[a] Equal aliquots of unlabled histone deacetylase complex were incubated with added [^{3}H]acetate-labeled histones for 2 h at 22 °C. The reaction was stopped by boiling for 1 min, and the free acetate was extracted and counted.

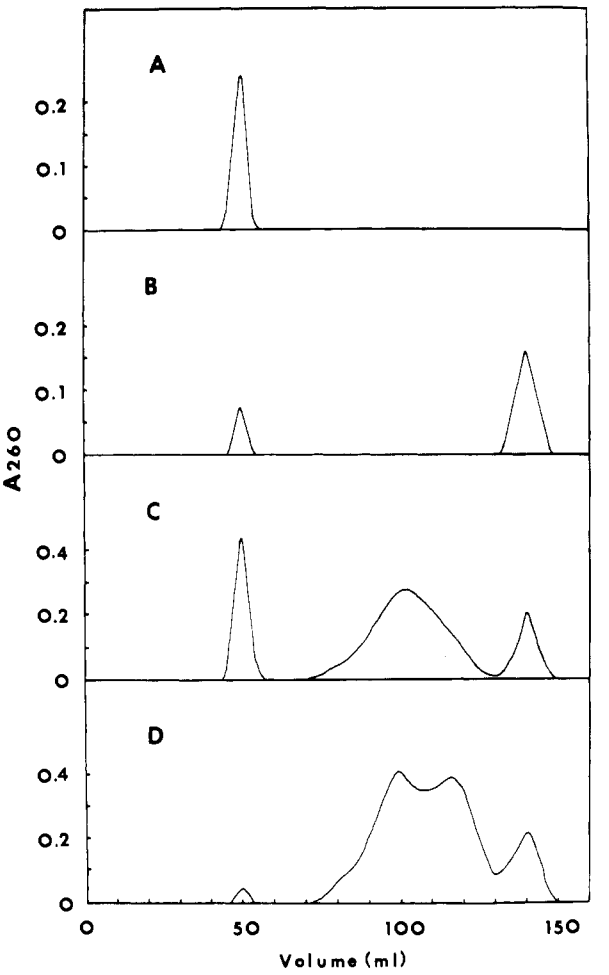

FIGURE 1: Fractionation of β-mercaptoethanol-treated histone deacetylase complex on a Bio-Gel A-50m column. (A) Histone deacetylase complex, purified by Bio-Gel A-50m chromatography, was rerun on a Bio-Gel A-50m column. (B) Histone deacetylase complex was applied to the same column containing 10 mM β-mercaptoethanol. (C) HeLa cell nuclei were partially digested with micrococcal nuclease by using standard procedures (Hay & Candido, 1983), and the histone deacetylase complex and nucleosomes were isolated by using a Bio-Gel A-50m column. (D) HeLa cell nuclei were isolated and partially digested with micrococcal nuclease as in (C) except that 10 mM β-mercaptoethanol was present. The histone deacetylase complex and nucleosomes were analyzed on the same column, with no β-mercaptoethanol present.

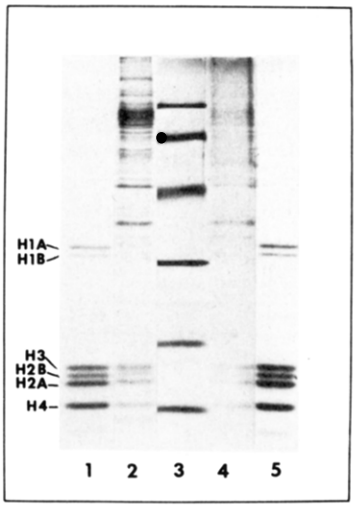

FIGURE 2: SDS–polyacrylamide gel profiles of histone deacetylase complex. The 15% gels were run according to Laemmli (1970) and were stained with Coomassie Blue. (Lane 1) HeLa histones; (2) HeLa histone deacetylase complex; (3) molecular weight markers (97.4K, 67K, 43K, 30K, 20.1K, and 14.4K); (4) HeLa histone deacetylase complex isolated from a Bio-Gel A-50m column containing 10 mM β-mercaptoethanol; (5) HeLa histones.

buffer during the second column run, two peaks were observed (Figure 1B). Again, quantitative recovery of the A_{260}-absorbing material was obtained, but the peak corresponding to the histone deacetylase complex was greatly reduced. The missing material was accounted for by the second peak, which corresponded to material smaller than mononucleosomes. When the experiment was repeated with endogenous acetate-labeled histone deacetylase complex, no histone deacetylase activity was detected in either peak.

Partial digests of HeLa cell nuclei with micrococcal nuclease normally yielded a profile such as that shown in Figure 1C when separated on a Bio-Gel A-50 column. A description of the protein and DNA contents across these column profiles has been presented (Hay & Candido, 1983). Briefly, the excluded peak contains histone deacetylase complex; the large included peak contains a broad distribution of nucleosomes with polynucleosomes of 50–20 nucleosomes in length at the high molecular weight side and mononucleosomes at the low molecular weight side. Material smaller than mononucleosomes is present in the third peak.

The presence of 10 mM β-mercaptoethanol during the preparation of the HeLa cell nuclei and the digestion with micrococcal nuclease, but not during the column fractionation, resulted in an altered column profile as seen in Figure 1D. There was a pronounced decrease in the amount of histone deacetylase complex and a corresponding increase in the amount of mono- and oligonucleosomes. Thus, the extent of digestion of the material normally eluting in the excluded region of the column was much greater in the presence of β-mercaptoethanol. The most probable explanation for the altered column profile is that β-mercaptoethanol disrupts the histone deacetylase complex, making the associated chromatin more accessible to micrococcal nuclease, which then degrades it to mono- and oligonucleosomes more rapidly.

We have previously compared the protein compositions of HeLa cell nuclear matrix and histone deacetylase complex (Hay & Candido, 1983), and both were found to contain a large variety of non-histone proteins. Several of the major proteins reported to belong to the HeLa nuclear matrix (Detke & Keller, 1982) were observed in the gel patterns of both the nuclear matrix and the histone deacetylase complex. Figure

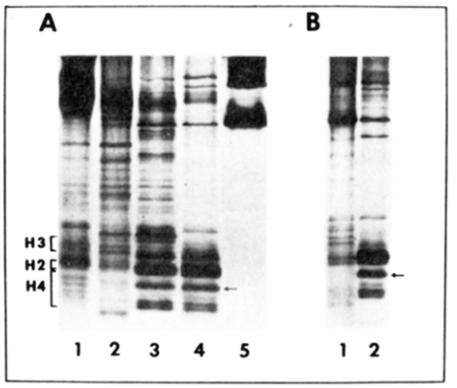

FIGURE 3: Acid–urea gel profiles of histone deacetylase complex and nucleosomes. Histones were prepared from histone deacetylase complex and nucleosomes as described under Experimental Procedures and applied to two acid–urea–15% polyacrylamide gels which were run according to the methods of Davie (1982). (A) (Lane 1) Histone deacetylase complex prepared from butyrate-treated HeLa cells; (2) histone deacetylase complex prepared from HeLa cells which had not been exposed to butyrate; (3) oligo- and mononucleosomes prepared from HeLa cells which had not been exposed to butyrate; (4) oligo- and mononucleosomes prepared from butyrate-treated HeLa cells; (5) bovine serum albumin and spermine which were used in the preparation of the histones (see Experimental Procedures). (B) (Lane 1) Histone deacetylase complex prepared from butyrate-treated HeLa cells and incubated at 22 °C for 2 h after removal of sodium butyrate by using the standard procedures for histone deacetylase assays; (2) oligo- and mononucleosomes from butyrate-treated HeLa cells.

2 shows a comparison of the SDS gel profile of intact histone deacetylase complex with that of the complex isolated from a Bio-Gel A-50m column containing 10 mM β-mercaptoethanol. A considerable enrichment of core histones is seen in the latter material. The dissociation of the complex in β-mercaptoethanol therefore seems to result in a significant loss of non-histone proteins.

Histone Content of Deacetylase Complexes. Histones were extracted from precipitated deacetylase complexes by displacement with 15 mM spermine, 6.25 M urea, and 0.9 M acetic acid (R. Kay, personal communication) and analyzed by acid–urea gel electrophoresis. Examination of the gels shown in Figure 3 shows that exposure of HeLa cells to 10 mM sodium butyrate for 8 h results in extensive hyperacetylation of the histones associated with the histone deacetylase complex. Gel scans revealed that tetraacetylated H4 was the most abundant form of histone H4 with slightly less tri-, di-, and monoacetylated H4. Very little (less than 5%) of the histone H4 was unacetylated. Comparison of this gel pattern with those of bulk histones from HeLa cells which have been exposed to butyrate (Candido et al., 1978) shows that the histones of the deacetylase complex from butyrate-treated HeLa cells were hyperacetylated to a greater degree than the bulk histones from these cells.

Examination of the histones from deacetylase complexes of cells which had not been exposed to butyrate also showed H4 to be hyperacetylated relative to bulk H4 from the same cells. The histones of the oligo- and mononucleosomes produced along with histone deacetylase complex during the micrococcal nuclease digest of HeLa cell nuclei were also examined and found to contain acetylated histones. The major forms of histone H4 in the oligo- and mononucleosomes were the unacetylated and monoacetylated derivatives. A protein with a molecular weight of approximately 12 000 (arrow, Figure 3) was observed in nucleosomes but not in preparations of the histone deacetylase complex. The size of this component was determined by two-dimensional gel electrophoresis with acid–urea gel electrophoresis in the first dimension and

SDS–polyacrylamide gel electrophoresis in the second dimension.

When histone deacetylase complex containing hyperacetylated histones was incubated at 22 °C for 2 h to allow deacetylation to take place, the resulting histones, analyzed by acid–urea gel electrophoresis, contained predominantly unacetylated and monoacetylated H4 (Figure 3B). Incubation of oligo- and mononucleosomes under the same conditions failed to alter the degree of histone acetylation. These results confirm our conclusions from other experiments that histone deacetylase is associated with a high molecular weight complex (Hay & Candido, 1983).

Restriction Enzyme Analysis of the Histone Deacetylase Complex DNA. The DNA associated with the deacetylase complex was found to range from 5 to 11 kbp, permitting restriction endonuclease analysis to be carried out. The human genome has a number of satellite DNA components which produce fragments of characteristic sizes when digested with particular restriction endonucleases. Since the sizes of the fragments created by the digestion of human satellites II and III with *Hae*III and *Eco*RI have been published (Mitchel et al., 1979), we digested histone deacetylase complex DNA and total HeLa cell DNA with these enzymes, as well as with *Taq*I and *Msp*I, in order to ascertain if these satellite sequences were significantly enriched or depleted in the histone deacetylase complex.

DNA was prepared from both the deacetylase complex and the HeLa cell nuclei by phenol extraction as described previously (Hay & Candido, 1983) and digested with the above restriction enzymes. The digestion products were separated by electrophoresis on 0.75% agarose gels, and the results are shown in Figure 4. *Hae*III digestion of either histone deacetylase complex DNA or genomic DNA resulted in a smear on which were superimposed several prominent bands. These bands had approximate sizes of 1170, 1050, 830, 710, 530, and 400 bp and correspond to the fragments produced by *Hae*III digestion of human satellite III. Digestion of histone deacetylase complex DNA and total DNA with *Eco*RI also produced a smear and bands of approximately 1720, 1400, 1070, and 780 bp. The largest two were the same size as the fragments produced by *Eco*RI digestion of human satellites II and III. The *Taq*I and *Msp*I digests of DNA from histone deacetylase complex and from HeLa nuclei yielded smears with no obvious bands.

Close scrutiny of the gel patterns of the *Hae*III and *Eco*RI restriction digests of histone deacetylase complex DNA and HeLa genomic DNA failed to detect any significant differences in the proportion of satellite DNAs. The DNA associated with the deacetylase complex is therefore not noticeably enriched or depleted in satellites II and III and appears to be of high complexity.

Conclusions

Treatment of endogenous labeled histone deacetylase complex with 1 or 10 mM β-mercaptoethanol caused both an irreversible inhibition of histone deacetylase activity (Table I) and the dissociation of the complex itself (Figure 1). The inhibition of deacetylase activity was probably not due to the reduction of thiol groups on the enzyme itself, since 10 mM β-mercaptoethanol did not affect the deacetylase activity of the complex when this was assayed with added histones (Table II). Also, neocuproine, which should have no effect on thiol groups, inhibited histone deacetylase activity in the endogenously labeled histone deacetylase complex to the same extent as β-mercaptoethanol. Thus, treatment of the deacetylase complex with reagents which disrupt chromosome scaffolds also leads to at least partial disruption of the complex and to a loss of deacetylase activity.

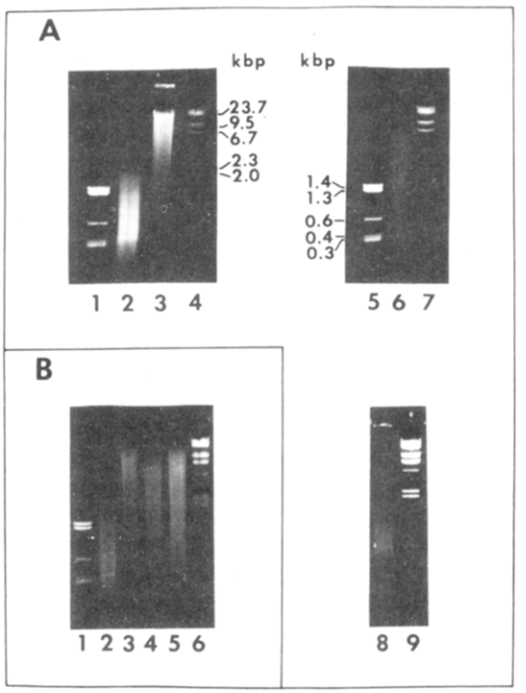

FIGURE 4: Restriction enzyme digestion of histone deacetylase complex DNA and genomic DNA. HeLa histone deacetylase complex DNA and HeLa genomic DNA were digested with several restriction enzymes. The digest products were analyzed by electrophoresis on horizontal slab gels of 0.75% agarose in Tris–borate buffer (pH 8.3). These were stained with 5 μg/mL ethidium bromide, and the DNA bands were visualized under UV light. (A) Histone deacetylase complex DNA: (lane 1) *Taq*I digest of pBR322; (2) *Hae*III digest; (3) *Eco*RI digest; (4) *Hin*dIII digest of λ DNA; (5) *Taq*I digest of pBR322; (6) *Msp*I digest; (7) *Hin*dIII digest of λ DNA; (8) *Taq*I digest; (9) *Hin*dIII digest of λ DNA. (B) HeLa genomic DNA: (lane 1) *Taq*I digest of pBR322; (2) *Hae*III digest; (3) *Eco*RI digest; (4) *Taq*I digest; (5) *Msp*I digest; (6) *Hin*dIII digest of λ DNA.

The digestion of HeLa nuclei with micrococcal nuclease in the presence of 10 mM β-mercaptoethanol resulted in a dramatic reduction in the deacetylase-containing peak of the Bio-Gel A-50m column, and an increase in the proportion of mono- and oligonucleosomes (Figure 1). This suggests that the deacetylase complex is disrupted by β-mercaptoethanol and implies that the histone deacetylase and associated nucleosomes form part of a large structure which is sensitive to β-mercaptoethanol or neocuproine. Such a complex, the chromosome scaffold, has been described by Lewis & Laemmli (1982). The chromosome scaffold dissociates upon treatment with β-mercaptoethanol or 3 mM neocuproine and seems to be maintained by metalloprotein interactions involving Cu^{2+} ions.

Restriction enzyme digestion of histone deacetylase complex DNA (Figure 4) showed that satellites II and III were not enriched in this material. Similarly, reannealing studies of the short DNA fragments anchored to the nuclear matrix of rat and mouse liver interphase nuclei and to the metaphase scaffold of Chinese hamster DON cell nuclei demonstrated that this DNA has the same complexity as genomic DNA and is not enriched in either repetitive or unique sequences (Basler et al., 1981).

In these studies, we have observed a high degree of acetylation in the histone H4 associated with the deacetylase complex from butyrate-treated HeLa cells (Figure 3). Thus, in vivo, the deacetylase complex is likely to be a site of very rapid acetyl group turnover, a notion which is consistent with the

rapid kinetics of deacetylation observed in vitro with this material (Hay & Candido, 1983). Perry & Chalkley (1982) have proposed that the regular acetylation and deacetylation of histones could allow chromatin to be decondensed for a variety of cellular functions.

An attractive working hypothesis at this stage is that histone deacetylase may be attached to a large matrix (possibly the chromosome scaffold) located at the base of chromatin loops. This would account for the high molecular weight of the deacetylase complex we observe, and also for the finding that the enzyme does not reversibly dissociate during the course of its reaction (Hay & Candido, 1983). The enzyme might then encounter its substrate nucleosomes in a processive manner. The high molecular weight histone deacetylase complex outlined in this report may thus provide a useful system to help elucidate the functions of histone acetylation and possibly of higher order nuclear structures.

Registry No. Histone deacetylase, 9076-57-7; β-mercaptoethanol, 60-24-2; neocuproine, 484-11-7.

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Accurate Molecular Weight Determinations of Deoxyribonucleic Acid Restriction Fragments on Agarose Gels†

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ABSTRACT: The electrophoresis of various DNA restriction fragments ranging in size from 47 to 6000 base pairs has been examined as a function of agarose concentration, electric field strength, and time of electrophoresis. A typical sigmoidal curve was obtained when the logarithm of the molecular weight was plotted as a function of mobility. The logarithms of the mobilities of all fragments were a linear function of gel concentration, if the mobilities of fragments ≥1000 base pairs were first extrapolated to zero electric field strength. The slopes of the lines, called the retardation coefficients, were found to be linearly proportional to the effective hydrodynamic surface areas of the fragments, as predicted by the Ogston theory of pore size distribution. The logarithm of the mobility of native DNA fragments was inversely proportional to $M_r^{0.8}$ over the entire molecular weight range, if the mobilities of fragments larger than 1000 base pairs were first extrapolated to zero electric field strength. The logarithm of the mobility of denatured, single-stranded DNA molecules was inversely proportional to the square root of molecular weight. The agreement of these results with the Ogston theory argues against a reptation mechanism for the movement of DNA molecules ≤6000 base pairs through agarose gels.

Despite the importance of gel electrophoresis in the preparation and purification of DNA restriction fragments, very little is known about the actual mechanism of the migration of DNA fragments through the gel. Obviously, molecular sieving plays an important role, since the diffusion coefficients of double-stranded DNA molecules in free solution are independent of molecular weight (Olivera et al., 1964). Lerman & Frisch (1982) have suggested that DNA molecules migrate through polyacrylamide gels by means of a reptation mechanism (deGennes, 1971). An equation describing such a wormlike or snakelike migration has been derived by Lumpkin & Zimm (1982).

The classical theory of gel electrophoresis, based on the Ogston model for a random meshwork of linear fibers (Ogston, 1958), predicts that the electrophoretic mobility of a macro-

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