Partial Characterization of the Histones and Histone Acetylation in Cell Cultures

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SUMMARY

In order to define the role of histone acetylation in cell cultures, a simple rapid method was developed to fractionate and quantitatively measure these proteins from milligram quantities of HeLa cells.

Histones were extracted from purified HeLa cell nuclei and resolved by electrophoresis in 0.1% ethylene diacrylate-15% acrylamide gels to fractionate 5 major histone components. The protein concentration in each histone band was measured quantitatively by densitometry of stained gels and radioactivity was determined by scintillation counting of sliced, base-hydrolyzed gels.

HeLa cell histones that were fractionated by differential extraction, precipitation, and Sephadex G-75 chromatography were found to be electrophoretically similar to and almost identical with calf thymus histones by amino acid analysis.

Cells grown in tritiated amino acids gave 4 radioactive peaks in acrylamide gels (the F2b and F2a2 were not resolved by this procedure). In contrast, cells pulsed with acetate-1.4C for either 30 or 45 min incorporated acetate into 3 peaks, which were identified by coelectrophoresis with amino acid-3H-labeled histone as the F3, the F2b + F2a2, and the F2a1. The fact that the acetate-1.4C-labeled histone fractions were consistently displaced toward the cathode in relation to amino acid-3H-labeled histone is tentatively interpreted as evidence for the loss of some of the effective positively charged e-NH$_3^+$ groups of the acetylated lysine residues.

INTRODUCTION

The biological role of histones is not well defined. The original hypothesis of Stedman and Stedman (43) that histones interact with DNA in a specific manner to control genetic expression was in doubt in view of the lack of significant qualitative differences both among species and among various tissues (9, 10, 15, 19, 25). Recently, however, evidence for heterogeneity in the very lysine-rich F1 (2, 3, 24) and certain biochemical modifications within the major histone fractions have reestablished a means of specificity for these basic nuclear proteins.

Disulfide interchange of the arginine-rich F3 histone fraction (14, 29, 30), methylation of the e-amino groups of lysine (45), phosphorylation of the serine (28), and acetylation (1, 11, 36, 46) of the histones have been observed in various systems. These activities appear to be under different controls. The disulfide form of the F3 histone is predominant in repressed chromatin such as unfertilized sea urchin eggs, whereas increasing amounts of free sulphydryl are found after fertilization (30). Histone phosphorylation appears to be associated with DNA synthesis in regenerating rat liver (28, 29). The relevance of the histone methylation activity in the regenerating rat liver is unknown (45).

The initial observations (1, 36) on the acetylation of histones during the early response of cultured lymphocytes to phytohemagglutinin suggested a relationship between histone acetylation and the subsequent stimulation of RNA synthesis. Differences in the pattern of acetate uptake and turnover between normal and regenerating rat liver have been observed in which maximal histone acetylation precedes the peak of RNA synthesis associated with partial hepatectomy (10, 38). Histone acetylation in regenerating rat liver was more active in the arginine-rich fractions (F3, F2a2, and F2a1) than in the lysine-rich fractions (F2b, F1); the difference in acetylation in the regenerating rat liver was manifested as a decreased rate of deacetylation in the F2a1 and F2a2 proteins (38). Treatment of acetate-1.4C-labeled histones with neutral hydroxylamine suggested that both the N-acetyl and O-acetyl groups were found in the F3 fractions, whereas only N-acetyl linkages were present in the F2a1 histone fraction (11).

The significance of these chemical modifications of histones is emphasized by the fact that DNA-dependent RNA synthesis in vitro was increased by a higher degree of acetylation (1) or phosphorylation (44) or by decreased disulfide in the F3 (13). These results therefore confirm that biochemical modifications of certain histone fractions can in general alter the RNA polymerase activity of the chromatin.
Cell cultures were selected for the study of histone acetylation because they permit controlled growth conditions. It was necessary, however, to devise a technique for the quantitative assay of histone acetylation utilizing small numbers of cells. This was accomplished by some modifications of existing procedures to permit analytical determinations of small amounts of reasonably pure histone fractions. These techniques were used for the preliminary characterization of the site of histone acetylation which is presented in this paper.

MATERIALS AND METHODS

Cell Culture. Reuber minimal deviation hepatoma cells (strain H4-II-E-C3), kindly supplied by Dr. Van R. Potter, and HeLa S-3 cells were routinely cultured in monolayer in minimal Eagle’s medium (7) in Hanks’ salts (12) supplemented with 10% calf serum (Microbiological Associates, Bethesda, Md.). Experiments were performed on monolayer cultures (1 to 2 × 10^7 cells) in 250-ml plastic culture flasks (Falcon Plastics Co.).

Radioactive Labeling. In different experiments histones were uniformly labeled with arginine-^14C or lysine-^14C (Uniformly labeled, 200 to 300 mCi/mM, New England Nuclear Corp., Boston, Mass.) or with phenylalanine-^3H (3 Ci/mM, New England Nuclear) by the addition of 0.1 μCi/ml ^14C (or 2 μCi/ml ^3H) to 2 successive 24-hr medium changes (approximately 2 generation times). Sodium acetate-1^14C (47 to 62 mCi/mM, New England Nuclear) was added (2 μCi/ml) directly to the cultures for the time indicated. At least 4 flasks were used for each determination. The cells were removed into 0.2% Triton X-100 and frozen in Dry Ice-ethanol to terminate the experiment.

Histone Extraction and Fractionation

Extraction of Total Histone. The cells were swollen in 0.2% Triton X-100 (20) and homogenized (4 to 6 strokes in a Dounce tissue grinder); a 4 times concentrated homogenization medium was added to make the normal concentration (0.25 M sucrose; 0.05 M Tris, pH 7.6; 0.005 M magnesium acetate; and 0.025 M KCl). After 4 to 6 additional strokes, the suspension (30 ml) was layered over a discontinuous sucrose gradient [7 ml 1.95 M sucrose (1.5 mM Ca++) and 5 ml 1.60 M sucrose (1.5 mM Ca++)]. and centrifuged 20 min at 13,000 × g in a Sorvall HB-4 rotor. The drained nucleic acid was resuspended in 20 ml 0.15 M NaCl-0.015 M sodium citrate, pH 6.5, and then centrifuged 10 min at 1,000 × g. The resulting pellet of washed nuclei was extracted at 4° in 8 ml 0.25 N HCl for a minimum of 4 hr. The solubilized histone was precipitated with 15% TCA, and washed with acidified acetone (21) and ether. The resulting white pellet was dissolved in 0.5 to 1.0 ml 1:10 diluted electrophoresis buffer plus 5% sucrose; 3 to 6 × 10^7 cells yield 2 to 4 mg of this crude total histone.

Purification of HeLa Histone Fractions. HeLa cells, 2.4 g (wet weight), were accumulated from monolayer cultures and stored at −18°. Nuclei washed in 0.15 M NaCl-0.015 M sodium citrate, pH 6.5, were prepared as described with the exception of an additional initial 800 × g 10 min centrifugation of the original homogenate before the sucrose gradient. The F2a and F3 histone fractions were isolated by slight modifications of the methods of Johns (21). Guanidine hydrochloride, 40% (w/v) (J. T. Baker Chemical Co., Phillipsburg, N. J.), was diluted with ethanol (1:3, v/v) without adjusting the pH of the solution. The 80% ethanol-washed nuclei were extracted at 4° with 20 ml followed by 10 ml, and the supernatants were combined for subsequent fractional acetone precipitation. The residue was extracted twice with 15 ml 5% PCA to solubilize the lysine-rich F1 histone. The residue was finally extracted with 10 ml 0.25 N HCl to recover the slightly lysine-rich F2b fraction. Both the PCA and HCl extracts were precipitated with 15% TCA, washed with acidified acetone and ether, and redissolved in 1.0 ml 0.01 N HCl.

Fractional Acetone Precipitation of the Argininc-rich Fractions. To the ethanol-guanidine hydrochloride extract, 1.1 volumes of acetone were slowly added with stirring at room temperature. The precipitate (F3 + F2a2) which formed after standing 12 hr at room temperature was recovered by centrifugation. Additional acetone was added to a total of 3 volumes, with the additional precipitate (F2a1) also recovered by centrifugation after standing for 12 hr at room temperature. Both fractions were washed with acidified acetone and ether and dissolved in 1.0 ml 0.01 N HCl.

Alternate Preparation of F1 for Amino Acid Analysis. Lysine-rich F1 was obtained in larger quantity by the 5% PAC extraction of purified nuclei (21). Contaminants consisting of minor amounts of other histone fractions were subsequently removed by Sephadex G-75 gel filtration.

Sephadex G-75 Chromatography. Columns (1.0 × 150 cm) of G-75 bead type Sephadex (Pharmacia Fine Chemicals, Inc., Piscataway, N. J.) were poured and washed with 0.01 N HCl, in which all samples were also dissolved and eluted (17). A 70-cm head maintained a 14 to 15 ml/hr flow rate for more than 12 preparation runs. The effluent was monitored at 235 mλ in a 1-cm (0.5-ml) flow cell in a Gilford Model 240 recording spectrophotometer and was collected in 12-drop (~0.8-ml) fractions. Pooled peak fractions were precipitated with 10% TCA, washed with acidified acetone and ether, and dried.

Alternate Histone Fractionation Procedure. NaCl, 0.15 M-0.015 M sodium citrate, pH 6.5, and 80% ethanol-washed nuclei from ~6 × 10^9 HeLa cells labeled with phenylalanine-^3H were fractionated according to the differential extraction Scheme 1 of Johns (21). The nuclei were successively extracted with 5% PCA, 1.25 N HCl: ethanol (1:4, v/v), and 0.25 N HCl. Each fraction was precipitated, washed with acidified acetone and ether, and resuspended in 1:10 diluted electrophoresis buffer. No further purification was attempted.

Histone Characterization

Amino Acid Analysis. Purified protein fractions (1 to 2

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4The abbreviations used are: TCA, trichloroacetic acid; PCA, perchloric acid.
mg) were dissolved in 1.0 ml 6 N HCl, and hydrolyzed at 107° ± 2° in a vacuum for 21 hr. The hydrolysates were evaporated to dryness and redissolved in 4.0 ml 0.01 N HCl. Analyses were performed on a Beckman C-120 amino acid analyzer.

Protein, RNA, and DNA Estimation. Protein was estimated by the method of Lowry et al. (26), with bovine serum albumin IV as the reference standard in all experiments. RNA was estimated by the orcinol method (4) and DNA was estimated by a modified indole procedure (41).

Acrylamide Gel Electrophoresis. The urea gel system of Reisfeld et al. (39) was used with the following alterations. In both 15% and 20% acrylamide gels, ethylene diamine tetraacetic (5) was added to a final concentration of 0.5% in the spacer gel and 0.1% in the running gel, instead of using bisacrylamide as the cross-linking agent. The electrophoresis buffer (71 mM β-alanine-34 mM acetate, pH 4.4, 2 liters/chamber) was cooled to 4° before each electrophoresis. The current was kept constant at 4 ma/tube for 2.25 hr for the 15% gel or 3.25 hr for the 20% gel. The gels were stained in 0.5% Amido black 10B (Harleco, Philadelphia, Pa.) in 7% acetic acid for 1 hr for analytical runs or for 6 hr for quantitative analyses. Destaining was accomplished by shaking the gels with several changes of 7% acetic acid.

Quantitative Determination of the Stained Histone Bands. Purified samples of the F1, F2b, and F2a1 HeLa cell histones were processed by electrophoresis at several concentrations and the destained gels were scanned at 615 mÅ with the use of the Gilford 240 gel scanner attachment. The tracings were measured quantitatively by planimetry (22).

Assay for Radioactivity in the Acrylamide Gels. After being treated by electrophoresis, 15% acrylamide-0.1% ethylene diamine tetraacetic gels were frozen on Dry Ice. They were sliced with stacked razor blades into 1-mm discs beginning at the interface between the spacer and running gels. Each slice was put in a scintillation vial with 1.0 ml 0.2 N NaOH and hydrolyzed for 36 hr at 37°. The resulting viscous solution was adjusted to pH 6 with 1 N acetic acid and counted with 15 ml Triton X-100-toluene-PPO-POPOP solution (32) in a Packard Model 3320 scintillation spectrometer. 14C and 3H efficiencies in this system are 80 and 19%, respectively.

RESULTS

The composition of the isolated HeLa nuclei was 87.5 weight % protein, 7.0% RNA, and 5.5% DNA, which is in agreement with those of Higashi et al. (13) who reported the composition of tumor cell (Walker 256 carcinosarcoma) nuclei as 82% protein, 11% RNA, and 7% DNA. Although one-third of the total nuclear protein was extracted with 0.25 N HCl, only barely detectable quantities of RNA and DNA contaminated this extract. Subsequent reextraction with 0.25 N HCl increased the yield of protein only 5%.

For identification of the proteins observed on acrylamide gels, purified calf thymus histone fractions (the gift of Dr. L. S. Hnilica) were compared to total HeLa cell and rat liver histone preparations, which are shown as densitometer tracings in Chart 1A and B. Dilute acid extraction of whole histone from nuclei always yielded minor amounts of non-

Chart 1. Spectrophotometric tracings of acrylamide gels. Fifteen % acrylamide gels were used for the qualitative analysis of total histone extracts from different sources. F2b and F2a2 could be partially resolved by the use of 20% acrylamide gels (D). A, 80 µg rat liver total histone; B, 80 µg HeLa cell total histone; C, 11 µg purified calf thymus F1; D, calf thymus F2b (16 µg) + F2a2 (15 µg); E, 15 µg calf thymus F2a1 plus F2a2.
histone protein in the anodic portion of the gels (Chart 1A and B). The fastest-migrating histone protein on acrylamide gel electrophoresis was F2a1 (Chart 1D). The F2b was identified as the slower-migrating component of the partially resolved 3rd band (Chart 1D) by treating F2b with F2a2 by coelectrophoresis (shown in Chart 1D), F2b with F2a1, and F2b with F2a2 and F2a1 combined (not shown). The 2nd band of the total histone pattern was F1 (Chart 1C), which was distinguished by its characteristic light blue staining in addition to its electrophoretic migration. Since no purified calf thymus F3 was available, we have deduced by difference that the slowest-migrating band was the F3 histone fraction. A similar migration pattern was also reported by Fambrough and Bonner (8). Since F3 contains cysteine (34) and evidence for considerable disulfide interchange has been reported (8, 9), we elected to denote the electrophoretically slower aggregate form as F3a and the rapidly migrating monomer as F3b (which migrates with F2b, slightly slower than F2a2). Our evidence for 2 electrophoretically distinct forms of F3 in HeLa cell histone is presented in Chart 2. Oxidized F3a and reduced F3b were treated together by electrophoresis with an F2a2 histone marker selected because it does not contain cysteine (34). Although treatment with 0.1 M β-mercaptoethanol did not effect a complete reduction of the F3a to F3b, the F3a was decreased from 10% to 4% of the total absorbance. The 5 major histone fractions (F1, F2b, F2a1, F2a2, and F3) thus purified by differential extraction and precipitation (23) and subsequent exclusion chromatography (17) were then further characterized by amino acid analysis. A comparison of the amino acid composition of HeLa cell histone fractions with the analogous calf thymus fraction is presented in Table 1. This comparison emphasizes the great similarity between these different sources. The amino acids which are most characteristic of each fraction are underlined. It was possible to obtain by these techniques electrophoretically pure F1, F2a1, and F2b for quantitative measurement of the stained histone bands. Since the determination of εmax for each stained band (Chart 3) showed that all fractions except F1 absorbed 97 to 99% of their maximum at 615 μm, the gels were scanned at 615 μm, at which wavelength only F1 had to be corrected for quantitative comparison with the other bands. The linearity of the stain (Chart 3) appeared to exceed 20 μg/fraction. The different staining characteristics and the apparently higher extinction coefficient of the F1 are as yet unexplained. Since F2a1, F2a2, F2b, and F3 seemed to have similar staining properties, the standard curve determined for F2a1 and F2b was used for all bands except F1.

Chart 2. Effect of β-mercaptoethanol on partially purified F3. A mixture of F3 and F2a2 was saturated with air at pH 8 to promote disulfide formation (18) and one-half of the sample was removed and treated with 0.1 M β-mercaptoethanol at 37° for 30 min. The solutions were acidified to pH 3, 50 μg of each were treated by electrophoresis and stained, and the peaks were measured quantitatively by densitometry. The original mixture (aerated) had 23 arbitrary area units of F3a and 204 area units of F3b + F2a2 (—); the 0.1 M β-mercaptoethanol-treated mixture had 10 area units of F3a and 238 area units of F3b + F2a2 (——).

Chart 3. Quantitative determination of stained histone bands on acrylamide gels. The purified HeLa histone fractions F1, F2a1, and F2b were treated by electrophoresis on 15% acrylamide-0.1% ethylene diacrylate gels, stained with Amido black, destained for 4 days, and scanned at 615 μm. The area on the tracing was measured quantitatively by planimetry. The absorption maximum for each stained band was determined by blanking the spectrophotometer against the unstained lower portion of the gel at each wavelength. The εmax for the F3 and F2a2 histones was ascertained from gels of partially purified preparations.

A partial identification of the histone bands could also be accomplished by the assay of radioactive histone on hydrolyzed acrylamide gels. Total histone extracts from cells labeled with either arginine-14C or lysine-14C were treated by electrophoresis separately; the radioactivity profiles of the sliced gels are superimposed in Chart 4A. Four distinct peaks
Chart 4. A, identification of histone bands on a sliced and hydrolyzed acrylamide gel. Total histone extracts from hepatoma cells labeled for 2 generation times with either arginine-\(^{14}\)C or lysine-\(^{14}\)C were treated by electrophoresis on separate acrylamide gels (see "Materials and Methods"). Two thousand cpm of each label were applied to the gels (1.4 X 10^5 cpm/mg arginine-\(^{14}\)C histone, 7.0 X 10^4 cpm/mg lysine-\(^{14}\)C histone). The gels were sliced, hydrolyzed, and counted and the radioactivity profiles were superimposed on the same axes. Each slice was 1 mm thick beginning with the spacer gel (S). B, incorporation of acetate into HeLa cell histone. Fresh medium was replaced in 4 flasks of HeLa cells which had been growing for 12 hr since the previous medium change. The cultures were pulsed with acetate-\(^{14}\)C (2 \(\mu\)Ci/ml) for 30 min, and the total histone extract was prepared (specific activity, 2.4 X 10^4 cpm/mg). Sixty-seven \(\mu\)g were treated by electrophoresis on an acrylamide gel which was sliced and counted. About 80% of the radioactivity applied to such gels is normally recovered after corrections for background (30 cpm) and quenching (~6%) have been made. From 50% to 60% of the applied radioactivity is usually recovered in the 3 peaks (A, B, and C) in the histone region of the gel.

similar to the spectrophotometer tracings of stained gels (Chart 1A and B) were well resolved. The F1 peak was readily identified because of its large excess of lysine to arginine. Both F3 and F2a1 exhibited their characteristic abundance of arginine, while the unresolved F2b and F2a2 peaks gave an intermediate composition.

In contrast to the amino acid-labeled histone pattern (Chart 4A), the pattern obtained for acetate-\(^{14}\)C incorporation into the total histone (Chart 4B) showed only 3 peaks (A, B, and C). Although Chart 4B is the acetylated histone pattern from cells grown for 30 min, qualitatively similar patterns have been obtained for labeling periods of 5 to 90 min (48).
Patterns such as those on Chart 4A and B suggested that the acetylated peaks A, B, and C corresponded to the F3, F2b + F2a2, and F2a1 fractions, respectively.

For confirmation of this interpretation, HeLa cells were grown in high-specific-activity phenylalanine-3H for 2 generations to label the histones uniformly with a tritiated amino acid. The histones were then fractionated according to Johns' Scheme 1 (21) into the very lysine-rich F1, the arginine-rich F3 + F2a, and the lysine-rich F2b. The 3H-amino acid-labeled histone fractions were treated by electrophoresis with identical samples of acetate-1-14C-labeled histone and gave the results portrayed in Chart 5A, B, and C. Chart 5A shows that the very lysine-rich F1 did not correspond to any of the acetylated histone peaks. The results of the electrophoresis of the arginine-rich fractions F3, F2a1, and F2a2 were close to the acetate-labeled proteins (Chart 5B), as were the F2b (Chart 5C). It was noticed, however, that the acetate-labeled peaks did not coincide with the main histone fractions, which were represented by the 3H-labeled amino acid label. To discount the possibility that these results were due to some artifact of preparation or handling, HeLa cell cultures were grown in the presence of phenylalanine-3H for 2 generations and then exposed to acetate-1-14C for 45 min. The incorporation patterns were qualitatively identical, as shown by the electrophoretic pattern of the total histone extract (Chart 5D). In addition, the same skewed peaks of the acetylated histone were observed in separate hepatoma cell experiments (47).

Therefore it can be concluded that Peak A of the acetylated histones (Chart 4B) was F3, Peak B was a combination of F2b and F2a2, and Peak C was F2a1. Several duplicate gels were counted and identically stained gels were measured quantitatively as previously described. In this manner F3 was calculated to have a specific activity of 50,000 cpm/mg; F2b + F2a2, 17,000 cpm/mg; and F2a1, 22,000 cpm/mg.

DISCUSSION

Although the quantitative distribution of histones from different sources has shown some variability (18, 30), the individual proteins appear to be qualitatively similar (8, 19, 25). Although some electrophoretic systems seem to yield considerable molecular heterogeneity in a histone fraction (27, 40), current evidence indicates the existence of only a small number of histone proteins (31). For example, F2a1 has been purified to homogeneity and its amino acid sequence has been determined (6, 38, 42). F2a2 has also been purified and found to have the same NH₂-terminal sequence as the F2a1 (35, 42), and NH₂-terminal peptide analyses indicate the F2b may be composed of 2 similar proteins (16). The very lysine-rich F1, however, does seem to be a mixture of several very similar proteins which have been partially fractionated and characterized recently by Busán et al. (2, 3) and Kinkade and Cole (24). Disulfide formation in F3 is thought to be responsible for the reports of molecular heterogeneity in this arginine-rich fraction (9, 17).

In this characterization of histones from metazoan cells, no qualitative differences from the histones of rat liver or purified calf thymus fractions were observed by standard analytical gel electrophoresis (Chart 1). The striking resemblance between HeLa cell histones and those of other species is clearer from the comparison of amino acid compositions (Table 1). The contamination of the HeLa F3 prepared by these procedures by less basic proteins is apparent since the basic : acidic amino acid ratio for calf thymus F3 is 1.7, compared with 1.0 for the HeLa cell preparation. As well, the lower lysine : arginine ratio of HeLa F2a2 compared with calf thymus may represent a slight contamination with F3, which could be obscured on acrylamide gels due to its similar electrophoretic mobility.

Chart 5. 14C-Acetylated histone treated by electrophoresis with phenylalanine-3H-labeled histone. A, B, and C each have 800 cpm (50 µg) of acetate-1-14C-labeled (45-min pulse) total histone treated by electrophoresis with the following amino acid-3H-labeled fractions. A, 700 cpm (50 µg) 5% PCA-extracted protein (F1); B, 5000 cpm (56 µg) of the 0.25 N HCl-80% ethanol extract (F2a1, F2a2, F3); and C, 4000 cpm (48 µg) of the final 0.25 N HCl extract (F2b).

Total histone from HeLa cells grown in phenylalanine-3H for 48 hr and then pulsed with acetate-1-14C for 45 min (see "Materials and Methods") is shown in gel pattern D. Three thousand cpm 3H (8% efficiency) and 1800 cpm 14C (59% efficiency) were applied to the gel. The double-labeled gels were counted with 7% 3H efficiency and 50% 14C efficiency, which gave 10% 14C overlap in the 3H channel.
Table 1: Comparison of the amino acid composition of HeLa cells and calf thymus histone fractions

Amino acid analyses were performed on HeLa cell histone fractions purified by differential extraction and Sephadex G-75 chromatography (see "Materials and Methods"). The purity of each fraction was determined by quantitative acrylamide gel electrophoresis (see Chart 3): F1, 95%; F2a1, 95%; F2a2, 85%; F2b, 90%; and F3, 60 to 70%.

The results are expressed as moles/100 moles amino acids recovered; no corrections were made for hydrolytic losses. Amide was not determined.

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<th>F2a1</th>
<th>F2a2</th>
<th>F2b</th>
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*a* Calf thymus values are from the work of Hnilica (16), except for F2b (15). These values were corrected for the hydrolytic loss (10%) of serine.

*b*Italicized numbers refer to amino acids which characterize a specific histone.

The extraction and fractionation procedures used for the purification of the histone fractions were designed to allow the preparation of reasonably pure histone proteins with minimal steps to ensure maximal recovery. In addition, good electrophoretic separation (Chart 1) of the histones was achieved on acrylamide gels which were also used for protein quantitative determination (Chart 3) and the assay of incorporated radioactivity (Charts 4A and B and 5A to D).

Chart 4 identifies the 4 distinct amino acid-labeled histone bands which were resolved by slicing a base-labile acrylamide gel. HeLa cells exposed to acetate-14C for 30 min, however, exhibited only 3 bands of incorporation (Chart 4B). Before the acetylation phenomenon could be investigated in greater detail, it was essential to identify at least partially the active histone fractions. Double-labeled gels were treated by electrophoresis to compare amino acid-3H and acetate-14C-labeled histone. Although amino acid-3H-labeled proteins can be detected (Chart 5B, C, and D) in the upper (anodic) portion of the gels, substantial acetylation is evident only in the faster-migrating histone portion of the electrophoretogram. Although the F1 of human lymphocytes and rat liver has been shown by others to incorporate acetate during a short pulse (36, 37), the studies reported here show no trace of any turnover or incorporation of labeled acetate (Chart 5A) in the F1 histone from HeLa cells. In contrast, the other 4 histone fractions (Chart 5B and C) can be treated by electrophoresis with the acetate-14C. Although an exact quantitative estimation of the acetylation among the F3, F2a2, and F2b proteins is not possible with the present electrophoretic separation, nevertheless it is apparent that in HeLa cells the F3 always exhibits the highest specific activity. Studies on the fractionation of suitable quantities of acetate-14C-labeled histone as well as the kinetics and response of histone acetylation to different growth conditions are reported (47, 48).

The fact that metabolically active acetate-14C-labeled histones cannot be treated by coelectrophoresis with the stable amino acid-3H-labeled histones (Chart 5B to D) suggests that the process of acetylation may be responsible for changes in their electrophoretic migration. The separation of the amino acid-3H-labeled histone from the acetate-14C-labeled fraction indicates that only a small percentage of histones are acetylated at the site involved in the uptake of acetate-14C. These conditions are supported by the identification of e-N-acetyllysine in F2a1, F2a2, and F3, a species that would effectively decrease the positive charge on these proteins (46). Our evidence for these conclusions is best observed in the F2a1 (Chart 5B and C). Since 95 to 100% of the F2a1 is amino-terminally acetylated (6, 33, 35, 42), it is unlikely that turnover of the amino-terminal acetate would account for the charge difference. The acetylation activity must therefore primarily reside in a small fraction of the F2a1 protein which is acetylated in the e-amino lysine position (~1 mole % for the highest specific activity measured (48), assuming 1 acetyl-14C group/F2a1 molecule (6, 42)). Panyim and Chalkley (31) have calculated that a
difference of 1 charge on F2a1 could change its migration by 3% of its total migration distance; thus a 1-slice (1-mm) difference between the acetylated and nonacetylated forms of F2a1 (which migrates 30 to 35 mm in the present system) would appear reasonable. If these interpretations are valid, the experiments reported here provide evidence that the acetylation of histones in vivo affects their basicity, which would be expected to alter their interaction with DNA or with other chromosomal proteins involved in DNA transcription.

ACKNOWLEDGMENTS

We thank Mr. John A. Hooper for his help with the amino acid analyses and Mr. Oliver E. Brown for invaluable technical assistance.

REFERENCES


Partial Characterization of the Histones and Histone Acetylation in Cell Cultures

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