Sodium Butyrate Induced Structural Changes in HeLa Cell Chromatin†

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ABSTRACT: Postsynthetic modifications of core histones by treatment of HeLa S3 cells with 5 mM sodium butyrate lead to alterations in the structure of high molecular weight chromatin. Whole chromatin from butyrate-treated cells, which results in highly acetylated core histones, has an ellipticity $[\theta]_{282.5}$ of 3700 deg cm^2 dmol^{-1} (0.2 mM EDTA, pH 7.4) that is 1200 deg cm^2 dmol^{-1} less than chromatin from untreated HeLa cells, suggesting a more condensed structure. No difference in the circular dichroism spectra was observed in H1-stripped, high molecular weight chromatin obtained from control and butyrate-treated cells at low (0.2 mM EDTA, pH 7.4) ionic strength. Thermal denaturation profiles of high molecular weight chromatin were resolved into three transitions and exhibited a shifting of hyperchromicity from transition I to transition III, at a higher T_m, with butyrate treatment of HeLa cells, further indicating a more compact structure. Thermal denaturation profiles of H1-stripped chromatin were not affected by butyrate treatment. Ionic strength studies in the range of 0–5 mM NaH_2PO_4, 0.2 mM EDTA, pH 7.4, of high molecular weight chromatin exhibited a decrease in $[\theta]_{282.5}$ and a shifting of hyperchromicity from transition I to transition III with increasing ionic strength. Control high molecular weight chromatin was more sensitive to changes in ionic strength than its highly acetylated counterpart. These results suggest that acetylation of histones alone does not result in a change in histone–DNA interaction but other changes associated with butyrate treatment most probably cause a more condensed structure, of the fraction studied herein, which is mediated by H1 or other materials removed during stripping in 0.35 M NaCl.

The general organization of chromatin includes two each of the basic proteins H2A, H2B, H3, and H4 bound to 146 base pairs of DNA and identified as the nucleosome core (McGhee & Felsenfeld, 1980). An additional 60 base pairs of DNA act as the linker between adjacent nucleosomes and are associated with histone H1 in some as yet unknown manner. Postsynthetic modifications of these proteins have long been thought to influence chromatin structure and function. One such modification, acetylation, has stimulated interest as a mechanism for the regulation of gene expression (Ruiz-Carrillo et al., 1975; Johnson & Allfrey, 1978; Reeves & Cserjesi, 1979) and as a signaling mechanism for embryonic differentiation (Poupko et al., 1977).

Of the core histones only H2B, H3, and H4 are acetylated to any great extent (Johnson & Allfrey, 1978). The major in vivo acetylation sites are located on H3 at the ϵ amino group of lysine in positions 9, 14, 18, and 23 of the polypeptide chain (Candido & Dixon, 1972) and on H4 at positions 5, 8, 12, and 16 (Dixon et al., 1975). Hence, histone acetylation is not a random event in the cell but occurs at specific sites in the positively charged N-terminal part of these proteins.

Attempts to reproduce the pattern of acetylation seen in vivo have met with varied results. Chemical acetylation with acetic anhydride, in vitro, gave random sites of modification along the polypeptide chain (Tack & Simpson, 1979). This method has proven to be unreliable for modification of histones at the established in vivo sites. Another approach was based on the observation that butyrate was effective in acetylating both H3 and H4 in HeLa and Friend erythroleukemia cells (Riggs et al., 1977). This acetylation was later found to be due to the reversible inhibition of an endogenous deacetylase in Friend erythroleukemia cells (Candido et al., 1978), HeLa cells (Vidali et al., 1978a), and hepatoma tissue culture cells (Perry et al., 1979; Sealy & Chalkley, 1978a). In addition to this reversible inhibition of the deacetylase, butyrate has been shown to block DNA synthesis (Hagopian et al., 1977), arrest cells in the G1 phase of the cell cycle (Hagopian et al., 1977; Prasad & Sinha, 1976), and produce changes in morphology, growth rate, and enzyme activities of cells in culture (Prasad & Sinha, 1976). Recently, calcium-dependent phosphorylation of H3 (Whitlock et al., 1980) and dephosphorylation of H1 (D'Anna et al., 1980a) have been demonstrated in butyrate-treated cells. All of these alterations are completely reversed with the withdrawal of butyrate from the cell system.

Closer investigation of these alterations with functional probes leads to similar results. Comparisons of DNase I[1] digestion kinetics showed an increased rate of digestion of acetylated chromatin over the unacetylated control (Simpson, 1978; Nelson et al., 1978; Vidali et al., 1978a). This increased DNase I sensitivity was assumed to have its origins in a reduced binding strength of acetylated histones. Histones isolated from transcriptionally "active" chromatin (Reeves & Candido, 1979; Davie & Candido, 1978) were found to be enriched in the acetylated forms of H3 and H4. Again, the assumption was made that a reduced binding affinity of acetylated histones for DNA made it possible for RNA polymerase to transcribe this portion of chromatin.

Despite evidence to the contrary (Sealy & Chalkley, 1978b; Mathis et al., 1978; Lilley & Berendt, 1979), it appears that acetylation is an important regulatory mechanism of the in vivo structure of chromatin. Acetylation would then be expected to destabilize the histone–DNA complex. Circular dichroism studies of acetylated H4–DNA reconstitutes offer direct evidence that acetylated H4 is less effective in altering

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[1] Abbreviations: CD, circular dichroism; DNase I, deoxyribonuclease I; % H_T, percent of total hyperchromicity at thermal transition; NP40, Nonidet P-40; PBS, phosphate-buffered saline (150 mM NaCl, 3 mM KCl, 8 mM Na_2HPO_4, and 1.5 mM KH_2PO_4, pH 7.4); PMSF, phenylmethanesulfonyl fluoride; solution A, 10 mM NaCl, 10 mM Tris, 3 mM $MgCl_2$, 0.5% NP40, and 0.1 mM PMSF, pH 7.4; solution B, 10 mM NaCl, 10 mM Tris, 3 mM $MgCl_2$, and 0.1 mM PMSF, pH 7.4; Temed, *N,N,N',N'*-tetramethylethylenediamine; T_m, temperature at midpoint of thermal transition; Tris, tris(hydroxymethyl)aminomethane; EDTA, ethylenediaminetetraacetic acid; BSA, bovine serum albumin.

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DNA conformation than its unacetylated counterpart (Adler et al., 1974).

This study was undertaken to establish a structural relationship between histone acetylation and the many biologic events attributed to it. Circular dichroism and thermal denaturation were used as monitors of structural changes. HeLa S3 cells were grown in the presence of 5 mM sodium butyrate for 24 h and served as the source of acetylated high molecular weight chromatin. High molecular weight chromatin was utilized in this study to probe whether acetylation of histones may effect higher order structure. McGhee et al. (1980) have suggested that modification of histone tails might weaken interchromatosome interactions and destabilize the solenoid structure.

Materials and Methods

Cell Culture. HeLa S3 cells were obtained from the American Type Culture Collection, Rockville, MD, and maintained in suspension at 37 °C with Joklik's modified minimal essential media containing 10% calf serum (Grand Island Biological Co., New York) as previously described (Riggs et al., 1977). Cells were maintained in the logarithmic phase of growth [(2–6) × 10^5 cells/mL] and harvested in lots of approximately 10^9 cells each. For experiments involving acetylated chromatin, cells were grown for 24 h in 5 mM sodium butyrate (*n*-butyric acid; Sigma Chemical Co., St. Louis, MO) and harvested at the end of the time period. Cells were counted with a hemacytometer, and viability was determined by the trypan blue exclusion test (Wintrobe, 1974).

Cells were prepared for storage by the following method. The suspension solution was centrifuged at 1000 rpm in a Model PR-2 centrifuge (International Centrifuge Co.) and the supernatant media were decanted. Cells were washed twice in 100 mL of phosphate-buffered saline (PBS: 150 mM NaCl, 3 mM KCl, 8 mM Na_2HPO_4, and 1.5 mM KH_2PO_4, pH 7.4). The cell pellet was then pooled into a 10-mL volume. These cells were resuspended in 30 mL of PBS containing 10% glycerol (Fisher ACS grade) and stored at −4 °C. Cells grown in butyrate were stored in PBS supplemented by 10% glycerol and 5 mM sodium butyrate to ensure both viability and continued inhibition of endogenous deacetylases.

Isolation of High Molecular Weight Chromatin. (A) Isolation of Nuclei. The method used to isolate HeLa nuclei is a modification of the procedure of Vidali et al. (1978b). Frozen cells were thawed and washed at least twice with PBS to remove any residual growth media and glycerol. Centrifugation was at 5000 rpm for 10 min in a Sorvall RC-2 centrifuge equipped with an SS-34 rotor. The cell pellet was resuspended in about 30 mL of detergent solution A [10 mM NaCl, 10 mM Tris, 3 mM $MgCl_2$, 0.5% NP40 (BDH Chemicals Ltd.), and 0.1 mM PMSF (Sigma Chemical Co.; stock solution is 0.1 M in dry dioxane), pH 7.4] and allowed to swell for 15 min in an ice bath. After incubation, the suspension was homogenized by 20 strokes in a Dounce homogenizer. Centrifugation follows for 10 min at 5000 rpm, and the detergent steps are repeated on the pellet fraction. The resulting nuclear pellet can be cleaned of cellular debris and detergent by 3–5 washings with solution B (10 mM NaCl, 10 mM Tris, 3 mM $MgCl_2$, and 0.1 mM PMSF, pH 7.4).

The procedure used for the isolation of nuclei from cells treated with 5.0 mM sodium butyrate was the same as the above procedure with the addition of 5 mM sodium butyrate to PBS, solution A, and solution B.

(B) Nuclear Digestion and Isolation of High Molecular Weight Chromatin. Staphylococcal nuclease (EC 3.1.4.7) digestion was performed as described in Noll et al. (1975) with minor modification. The nuclear pellet was diluted to an A_{260} of 40 with digestion buffer containing 300 mM sucrose, 10 mM Tris, 0.75 mM $CaCl_2$, and 0.1 mM PMSF, pH 7.4. After a 5-min preincubation in a 37 °C water bath, the nuclear suspension was digested with 10 units/mL of staphylococcal nuclease (Worthington Biochemical Corp.) for 2 min. This amount of digestion corresponds to about 10% acid-soluble material. The nuclease reaction was quenched with 0.1 volume of 0.1 M EDTA, pH 7.4, and incubated on ice for 15 min to terminate the reaction. The nuclei were centrifuged for 15 min at 10000 rpm in the RC-2 centrifuge equipped with a SM-24 rotor. The supernatant was removed and corresponds to the low molecular weight fraction. The pellet was extracted with 10 volumes of 0.2 mM EDTA, pH 7.4, overnight at 4 °C. Centrifugation of this suspension and removal of the supernatant yields the high molecular weight fraction. So that the yield could be increased, 2–3 additional EDTA extractions were performed.

The fractions obtained were pooled and dialyzed overnight in column buffer (0.2 mM EDTA and 10 mM Tris, pH 7.4). The dialyzed material was concentrated to a volume of approximately 14 mL with a large Amicon concentrator equipped with a PM10 membrane filter (Amicon Corp., Lexington, MA). The resulting chromatin containing solution was applied to a Bio-Gel A-150m or A-15m column (Bio-Rad Co.) (2.5 cm × 55 cm) equilibrated with column buffer. This column separates HeLa chromatin into three fractions by molecular weight, high molecular weight material being coincident with the void volume (yield = 20–40%). When not in use, this column was stored in column buffer containing 0.02% sodium azide. The use of this column is a modification of the procedure cited in Fulmer & Fasman (1979a,b). Samples obtained in this manner were stored at 4 °C in column buffer for periods not to exceed 3 weeks.

(C) Stripping of H1 from High Molecular Weight Chromatin. High molecular weight chromatin from the void volume of the Bio-Gel A-150m column (12 mL, $A_{260} \simeq 2.0$) was dialyzed into 0.35 M NaCl column buffer. The chromatin was concentrated in a small Amicon concentrator with a PM10 membrane to a volume of 1 mL. The chromatin was passed through a Bio-Gel A-0.5m column (0.8 cm × 45 cm) equilibrated in 0.35 M NaCl column buffer. The void volume was pooled as the H1-stripped high molecular weight chromatin. The column was kept at 4 °C and stored in 0.35 M NaCl column buffer with 0.02% NaN_3 when not in use.

Gel Electrophoresis. The molecular weight of DNA isolated from high molecular weight chromatin was determined by electrophoresis on 4% polyacrylamide and 1.4% agarose slab gels by the method of Maniatis et al. (1975). Calibration of DNA mobility in these gels was by coelectrophoresis of *Hin*cII–*Hin*dIII (New England Bio-Labs) restriction fragments of λ DNA (Miles). Preparation of these gels is as described in Cowman & Fasman (1980).

Electrophoresis of Histone Proteins. Electrophoresis in polyacrylamide tube gels was as follows. Samples to be analyzed were lyophilized, and then the proteins were dissociated from DNA by dissolving in 8 M urea, 0.9 N acetic acid, 10 mg/mL protamine sulfate (Worthington Biochemicals), 1% 2-mercaptoethanol (Eastman), and 1 mM PMSF and heated for 30 min at 60 °C (Schaffhausen & Benjamin, 1976). Samples containing about 36 μg of DNA were dissolved in 50–70 μL of the protamine sulfate solution; thus the ratio of protamine sulfate to core histones was 500–700 μg:30 μg. Approximately 36 μg of protein obtained in this manner was loaded onto 26-cm tube gels containing 13% acrylamide, 0.34%

N,N'-methylenebis(acrylamide) (Bio-Rad), 2.5 M urea, and 0.9 N acetic acid (polymerization was started by adding TEMED to a final concentration of 0.5% and persulfate to a final concentration of 0.05%). This gel system, described by Traub & Boeckmann (1978), gave optimum separation of the acetylated forms of H3 and H4. The tube gels were preelectrophoresed overnight at 200 V in a buffer containing 0.9 N acetic acid. After the buffer had been changed, the samples were loaded and run at 180 V for about 20 h (1–1.5 mA/gel). The gels were stained with 0.1% amido black in 50% methanol and 7% acetic acid and destained by diffusion in the same methanol–acetic acid solvent. The gels were scanned at 570 nm in a Gilford Model 222 spectrophotometer equipped with a Gilford Model 6×2D linear transport and Fisher Recordall Series 5000 strip recorder. Areas of the stained histone bands were determined with a Du Pont 310 curve resolver.

DNase I Digestion Kinetics. Nuclei from control and sodium butyrate treated HeLa cells were prepared with the method described above. DNase I digestion was determined by a modification of the procedure of Simpson (1978). Parallel digestions were arranged to minimize any differences in the activity of DNase I (Sigma Chemical Co.) (EC 3.1.4.5) that may occur as a result of repeated freezing and thawing. Nuclei were suspended in digestion media (0.25 M sucrose, 10 mM Tris, and 10 mM $MgCl_2$, pH 8.0) to an $A_{260} \simeq 10.0$. The nuclei were preincubated for 10 min at 37 °C. At $t = 0$, 40 units/mL of enzyme was added to the nuclear suspension at 37 °C. Aliquots of the suspension were removed from the reaction mixture and quenched with 0.1 volume of 0.1 M EDTA, pH 7.0, at several time points from 0 to 60 min in duration. The quenched reaction was allowed to terminate in ice for 15 min and centrifuged at 12000*g* in an Eppendorf centrifuge. DNA in the supernatant was precipitated with 2 volumes of a PCA solution (0.8 M perchloric acid, 0.8 M NaCl, and 100 μg/mL BSA) and placed on ice for 20 min. This reaction mixture was again centrifuged in the Eppendorf centrifuge for 10 min. The absorbance at 260 nm of the resulting supernatants was read and acid solubility calculated as a percentage of the original nuclear suspension. The reported kinetics are the result of duplicate digestions on control and acetylated nuclei from two different preparations.

Physical Measurements. High molecular weight HeLa chromatin was prepared for analysis by dialyzing against 0.2 mM EDTA, pH 7.4. Ionic strength effects were evaluated in the above buffer supplemented by 0, 1, 3, and 5 mM NaH_2PO_4, pH 7.4. The concentration of chromatin was adjusted to an $A_{260} \simeq 1.0$ with dialyzate. Absorption spectra were recorded on a Cary 14 spectrophotometer at room temperature. Concentrations obtained in this way were corrected for light scattering effects.

(*A*) *Circular Dichroism.* All circular dichroism (CD) spectra were obtained at 23 °C with a Cary 60 spectrophotometer equipped with a 6001 circular dichroism accessory as described by Cowman & Fasman (1980). Samples were contained in a jacketed quartz cell with 1-cm path length (Optical Cell Co., Woodbine, MD) and scans performed in the wavelength range of 200–350 nm. DNA concentrations were expressed as per mole of nucleotide residue and determined by absorption at 258 nm [$\epsilon_{258} = 6800$ cm^{-1} (mol of nucleotide)$^{-1}$]. Instrument sensitivity was maintained at 0.02 deg for full-scale deflection. Reported spectra are averages of 4–6 spectra obtained on 2–3 separate preparations of high molecular weight HeLa chromatin.

(*B*) *Thermal Denaturation Analysis.* Parallel samples of high molecular weight chromatin that had been exhaustively dialyzed in 0.2 mM EDTA, pH 7.4, and supplemented with 0, 1, 3, and 5 mM NaH_2PO_4 were subjected to thermal denaturation analysis. The sample to be analyzed was warmed to room temperature and degassed by bubbling helium for approximately 5 min. This sample was then transferred to a 1-cm path length jacketed quartz cuvette (Hellma 1-160B QS) with a tight-fitting Teflon stopper. Denaturation was monitored as the change in absorbance at 260 nm with the Cary 14 spectrophotometer. Temperature in the cuvette was monitored with a linear thermal probe located adjacent to the point at which the circulant leaves the cuvette. Temperature increase was controlled to a rate of ~0.2 °C/min. Data acquisition, computer analysis, and derivative calculation are as previously described (Fulmer & Fasman, 1979a,b). The derivative dh/dT was resolved into transitions by Gaussian curve fitting on a Du Pont 310 curve resolver. The transition midpoint T_m of the derivative profile is defined as the temperature at a maximum dh/dT for each transition and was reproducible to ~2.0 °C. The total hyperchromicity was 32.5 ± 2.1 for both control and acetylated chromatin. The denaturation profiles reported here are averages of two to three profiles.

(*C*) *pH Measurements.* A Radiometer Model 25 pH meter with a Radiometer Type GK-2302 combination electrode was used to measure pH. Calibration was performed with standard buffers (Fisher Scientific).

Miscellaneous. All purification procedures were performed at 4 °C unless otherwise stated. Solutions were prepared at room temperature with glass-distilled water. The chemicals used throughout were reagent grade. Dialysis tubing was Spectrapor 1 and 3 (Spectrum Medical Industries) that had been pretreated by boiling in 10 mM $NaHCO_3$ and 1 mM EDTA for 60 min, followed by boiling for 60 min in glass-distilled water and liberal rinsing.

Protein concentrations were determined by the method of Lowry et al. (1951). Standard curves were generated with bovine serum albumin that had been carefully calibrated against purified H4 (correction factor = BSA (mg/mL) × 0.755). DNA concentration was determined by absorbance at 260 nm. $A^{1\,cm}_{258}$ of 20 = 1 mg/mL DNA.

Results

Characterization. (*A*) *DNA Molecular Weight Determination.* A variety of methods were used to ensure that the material used in this study was of high molecular weight and, in the case of butyrate-treated chromatin, sufficiently acetylated. DNA from control chromatin was found to be greater than 10^6 daltons on 4% polyacrylamide gels and greater than 1.6×10^6 daltons on 1.4% agarose gels. Similar electrophoretic patterns of high molecular weight DNA obtained from butyrate-treated chromatin gave a molecular weight in excess of 10^6 on 4% polyacrylamide gels and greater than 1.4×10^6 on 1.4% agarose gels. Determination of the protein:DNA ratio (g/g) for control chromatin was found to be 1.34, and for acetylated chromatin the ratio was 1.32 (see Materials and Methods).

(*B*) *DNase I Digestion Kinetics.* DNase I digestion kinetics were found to be different for control and acetylated nuclei. Control chromatin was digested to approximately 50% acid solubility by about 1 h of digestion time and remains at that level. Acetylated chromatin, digested under the same conditions, was digested to a maximum of about 70% acid solubility by 1 h. The overall rate and extent of digestion were higher in the case of acetylated chromatin than in its control counterpart (data not shown).

(*C*) *Histone Polyacrylamide Electrophoresis.* So that the

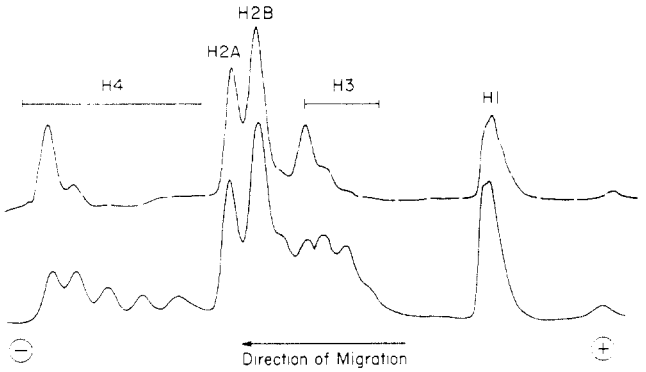

FIGURE 1: Electrophoretogram of histones from control and butyrate-treated high molecular weight chromatin from HeLa cells. Scans of acetic acid–urea–13% polyacrylamide gels. (--) Control chromatin; (—) chromatin from butyrate-treated cells.

extent of acetylation and the ratios of histones in control and highly acetylated chromatin could be determined, 13% polyacrylamide tube gel electrophoresis was performed on the chromatin by the methods of Schaffhausen & Benjamin (1976) and Traub & Boeckmann (1978). The gels of highly acetylated chromatin from butyrate-treated nuclei indicate that several additional bands in the H3 and H4 regions are present with mobilities consistent with histones of varying degrees of acetylation (Riggs et al., 1977). Scans of these gels (Figure 1) were performed and the area under the curves was resolved. In the control scans, H4 was found to be in two forms; the unmodified form accounted for 75% of the total H4 area, and the monoacetylated form represented 25% of the total area. The H3 peak was resolved into three peaks, unmodified, monoacetylated, and diacetylated, containing 60%, 26%, and 13% of the total H3, respectively. Highly acetylated chromatin was found to be much more extensively modified. H4 was found to contain unmodified, monoacetylated, diacetylated, triacetylated, and tetraacetylated bands representing 22.5%, 26.5%, 21%, 14%, and 16% of the total H4, respectively. Similarly, the H3 bands can be seen in the unmodified, monoacetylated, diacetylated, and triacetylated forms with 26%, 29%, 18%, and 27% of the total H3 area, respectively. However, because of difficulties in the resolution of the H3 area on these gels, the areas given as H3 may contain modified H2A and H2B species. The content of H1 was found to be essentially the same in control and acetylated chromatin, 14% and 17%, respectively, of the total histone area. There was a considerable loss of nonhistone proteins when isolating high molecular weight chromatin from nuclei by the procedures used herein (data not shown).

Circular Dichroism. Circular dichroism analysis of the chromatin as characterized above yielded the following. Increasing the ionic strength of the solvent led to a general decrease in the ellipticity at 282.5 nm. In control chromatin (Figure 2A), $[\theta]_{282.5}$ decreased from 4900 deg cm^2 (dmol)$^{-1}$ at 0 mM NaH_2PO_4 and 0.2 mM EDTA, pH 7.4, to 3800 deg cm^2 (dmol)$^{-1}$ at 5 mM NaH_2PO_4 and 0.2 mM EDTA, pH 7.4. The $[\theta]_{282.5}$ at 3 mM NaH_2PO_4 is the same as at 5 mM NaH_2PO_4. The largest change in ellipticity occurs when the ionic strength is varied in the range of 0–1 mM NaH_2PO_4. This demonstrates that the major transition occurs from 0 to 1 mM sodium phosphate. For acetylated chromatin (Figure 2B), the same general decrease in ellipticity as a function of increasing ionic strength is observed. At 0 mM NaH_2PO_4 and 0.2 mM EDTA, pH 7.4, $[\theta]_{282.5}$ is 3700 deg cm^2 (dmol)$^{-1}$. This value gradually decreases to 2800 deg cm^2 (dmol)$^{-1}$ at 5 mM NaH_2PO_4 and 0.2 mM EDTA, pH 7.4. The most prominent change can again be observed between 0 and 1 mM NaH_2PO_4

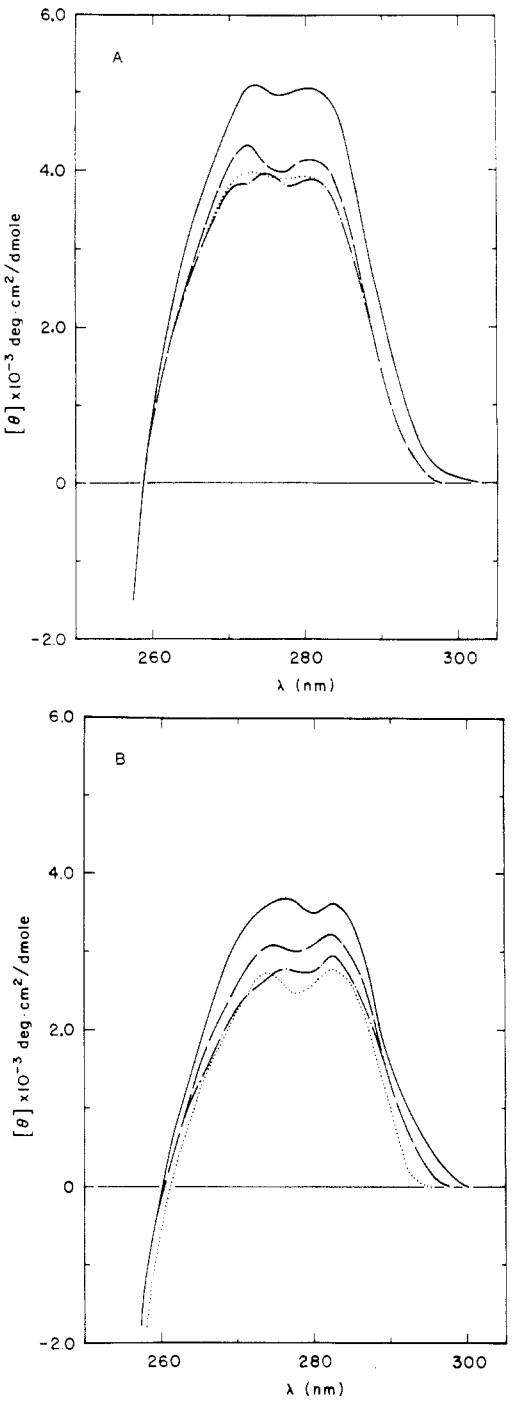

FIGURE 2: (A) Circular dichroism spectra of control chromatin as a function of ionic strength. (—) 0.2 mM EDTA, pH 7.4; (--) 1.0 mM NaH_2PO_4 and 0.2 mM EDTA, pH 7.4; (···) 3.0 mM NaH_2PO_4 and 0.2 mM EDTA, pH 7.4; (-·-) 5.0 mM NaH_2PO_4 and 0.2 mM EDTA, pH 7.4; cell path length 1 cm; OD_{260} of chromatin $\simeq$1.1; temperature 23 °C. (B) Circular dichroism spectra of acetylated chromatin as a function of ionic strength. (—) 0.2 mM EDTA, pH 7.4; (--) 1.0 mM NaH_2PO_4 and 0.2 mM EDTA, pH 7.4; (···) 3.0 mM NaH_2PO_4 and 0.2 mM EDTA, pH 7.4; (-·-) 5.0 mM NaH_2PO_4 and 0.2 mM EDTA, pH 7.4; cell path length 1 cm; OD_{260} of chromatin $\simeq$1.1; temperature 23 °C.

with small decreases in ellipticity at the higher ionic strengths. The crossover point was unaffected by ionic strength or extent of acetylation in the range investigated. A summary of these data is presented as Table I.

A significant difference in the CD spectra of the two samples can be seen in Table I. At 0 mM NaH_2PO_4 and 0.2 mM EDTA, pH 7.4, the spectra of high molecular weight chromatin is seen to be much smaller in magnitude for acetylated

Table I: Summary of Data from Circular Dichroism Studies

$[NaH_2PO_4]$ [a] (mM)	control chromatin			acetylated chromatin		
	$[\theta]_{272.5}$ [b]	$[\theta]_{282.5}$	crossover point (nm)	$[\theta]_{272.5}$	$[\theta]_{282.5}$	crossover point (nm)
0	5100 [c]	4900	259	3400	3700	260
1.0	4300	4100	259	3000	3200	260
3.0	3800	3800	259	2600	3000	261
5.0	4000	3800	259	2700	2800	261

[a] All solutions contain 0.2 mM EDTA, pH 7.4. [b] Ellipticity is expressed as deg cm^2 (dmol)$^{-1}$. [c] Values are ±300 deg cm^2 (dmol)$^{-1}$.

Table II: Transition Midpoints [a] and Relative Areas of Thermal Transitions [b] of Control and Acetylated Chromatin

$[NaH_2PO_4]$ [c] (mM)	transition I		transition II		transition III	
	T_m (°C)	$\%H_T$	T_m (°C)	$\%H_T$	T_m (°C)	$\%H_T$
Control Chromatin						
0	55.0	48	67.0	17	77.0	36
1.0	55.2	38	65.0	14	76.5	48
3.0	59.5	35	66.2	10	77.3	55
5.0	59.0	22	64.5	15	77.0	63
Acetylated Chromatin						
0	54.0	22	63.0	19	73.0	59
1.0	57.0	21	65.3	21	75.5	58
3.0	58.5	12	65.0	27	75.0	61
5.0	61.0	18	66.0	14	76.0	68

[a] T_m, temperature of maximum dh/dT for each transition, reproducible to ±2 °C. [b] $\%H_T$, percent of total hyperchromicity found in a given transition, reproducible to ±5%. [c] All chromatin solutions are in 0.2 mM EDTA, pH 7.4.

chromatin as compared to control chromatin; a difference of 1200 deg cm^2 (dmol)$^{-1}$ was found. A similar comparison of the spectra at higher ionic strength showed the same general trend of a lower ellipticity, $[\theta]_{282.5}$, of acetylated material compared to the control, but the effect was most pronounced at 0 mM NaH_2PO_4.

Thermal Denaturation Analysis. So that a more detailed picture of the structural changes resulting from acetylation of high molecular weight chromatin could be obtained, as indicated by circular dichroism, parallel samples were analyzed with thermal denaturation. Aliquots of high molecular weight chromatin, obtained as described above, were exhaustively dialyzed against solutions of 0.2 mM EDTA, pH 7.4, containing 0–5.0 mM sodium phosphate at 4 °C. Thermal denaturation profiles, dh/dT vs. T, of control chromatin are shown in Figure 3A–D and for acetylated chromatin in Figure 3E–H. The transition midpoints as well as the relative areas of the thermal transitions of both control and acetylated chromatin are given in Table II.

The thermal denaturation profiles of control chromatin in 0.2 mM EDTA, pH 7.4, displayed approximately 48% of the total hyperchromicity in transition I_C with a T_m of 55 °C. Transition II_C contained about 17% with a T_m of 67 °C, and transition III_C contained about 36% of the total hyperchromicity with a T_m at 77 °C. Increasing the sodium phosphate concentration to 1 mM reduced the percent of total hyperchromicity in I_C to about 38%, leaving the T_m at 55 °C. This decrease in $\%H_T$ of 10% can be found in III_C where the $\%H_T$ increases about 12%, the added contribution coming from a slight decrease in $\%H_T$ from II_C. The T_m for both II_C and III_C remained relatively unchanged. A small change in T_m, as seen in II_C, of about 2 °C is considered here to be within the range of experimental variability for the instruments and techniques used in this study. Increasing the sodium phosphate concentration further to 3.0 mM continued to show these

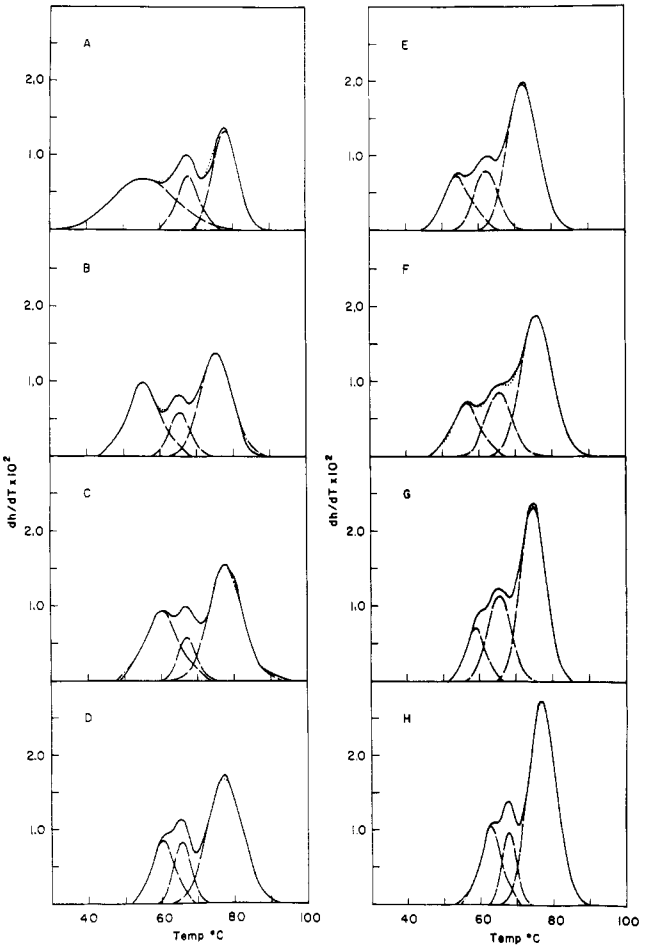

FIGURE 3: Thermal denaturation profiles, dh/dT vs. T, of control and acetylated high molecular weight chromatin as a function of ionic strength. Control chromatin (A–D). Acetylated chromatin (E–G). (A and E) 0.2 mM EDTA, pH 7.4; (B and F) 1.0 mM NaH_2PO_4 and 0.2 mM EDTA, pH 7.4; (C and G) 3.0 mM NaH_2PO_4 and 0.2 mM EDTA, pH 7.4; (D and H) 5.0 mM NaH_2PO_4 and 0.2 mM EDTA, pH 7.4; path length 1 cm; heating rate 12 deg/h.

general trends. A further decrease in $\%H_T$ in I_C of about 3% with a decrease of 4% in II_C expresses itself as an increase in the $\%H_T$ of III_C of 7%. In addition the T_m of I_C shifts about 4 °C higher at this ionic strength while the T_m for II_C and III_C remains unchanged. The final increase in ionic strength to 5.0 mM further lowered the $\%H_T$ in I_C to about 22%, a decrease of about 26% from the values obtained at 0 mM sodium phosphate. Resolution of transitions I_C and III_C becomes more difficult at this ionic strength since the T_m of transition I_C remains unchanged at about 59 °C but the T_m of transition II_C shifts about 3 °C lower to 64.5 °C. The $\%H_T$ value for II_C increases about 5% over that seen at 3.0 mM sodium phosphate but is well within experimental sensitivity of the values reported for 0 and 1.0 mM sodium phosphate. The T_m for transition III_C remains unchanged compared to all other ionic strengths tested, but the $\%H_T$ of this transition

increases further to about 63%, almost 10% larger than at 3.0 mM sodium phosphate and 27% larger than at 0 mM sodium phosphate.

The general trends observed for control chromatin indicate that as ionic strength increases from 0 to 5.0 mM sodium phosphate, there is a shift from transition I_C to III_C. Minor variations in this pattern exist, however, with a slight increase in T_m to about 59 °C for transition I_C at high ionic strength and a somewhat depressed area in transition II_C at 3.0 mM sodium phosphate.

Acetylated chromatin isolated from butyrate-treated cells shows a decrease in % H_T from 0 to 5.0 mM sodium phosphate in transition I_A accompanied by an increase in % H_T for transition III_A with increasing ionic strength but with notable differences compared to the control. A qualitative assessment of these changes is evident in Figure 3E–H. However, the pattern is somewhat more variable with ionic strength than that observed in control chromatin. At 0 mM sodium phosphate the T_m of transition I_A is 54 °C and the percent of total hyperchromicity for this transition is 22%. Increasing sodium phosphate concentration to 1.0 mM increases the T_m slightly, but % H_T for this transition remains the same. Further increases to 3.0 mM show the T_m remaining constant at about 58 °C, but the % H_T decreases 10% over that observed in I_A at 1.0 mM. Finally, increasing the ionic strength to 5.0 mM gives a small increase in T_m, but the relative area of I_A increases almost 6% over that seen at 3 mM and approaches the 22% value observed at 0 mM sodium phosphate.

Transition II_A varies somewhat with increasing ionic strength. A slight increase in the T_m from 63 to 65 °C is evident on increasing ionic strength from 0 to 1.0 mM sodium phosphate. This difference is within experimental variation and probably does not represent a real shift in T_m. However, the % H_T for II_A with ionic strength increases steadily from 0 to 3.0 mM sodium phosphate to a value almost 10% higher than that seen at 0 mM. This variable behavior in % H_T of II_A with ionic strength in the 3.0 mM sodium phosphate region is consistent with a conformational transition where even slight changes in ionic strength may alter structure. Further increases to 5.0 mM show a decrease in this transition. Part of this decrease may be attributed to the increase seen in I_A and part to the increase in III_A at this ionic strength.

Transition III_A shows a slight increase in T_m from 73 to about 76 °C at 1.0 mM sodium phosphate and remains constant during any further increases in ionic strength. In addition the % H_T in transition III_A undergoes an increase of about 7% during the increase from 3.0 to 5.0 mM sodium phosphate. Thus, in acetylated chromatin, two transitions appear to be present. The first is a transition in T_m upon increase of ionic strength from 0 to 1.0 mM sodium phosphate. The second is a change in the % H_T upon increasing the ionic strength from 3.0 to 5.0 mM sodium phosphate.

In summary, for acetylated chromatin the % H_T for transitions I_A, II_A, and III_A does not markedly change with increasing ionic strength except perhaps at 3.0 mM sodium phosphate. This is in contrast to the changes in control chromatin where a dramatic change occurs in both I_C and III_C as a function of ionic strength.

The most notable differences in the thermal denaturation data appear upon comparison of control and acetylated chromatin. At 0 mM sodium phosphate, for example, the percent of total hyperchromicity in transition I_A is 26% lower than that found in I_C, and the % H_T in transition III_A is about 23% higher than that found in III_C. An important difference in the T_m of transition III is noted at low ionic strength. The

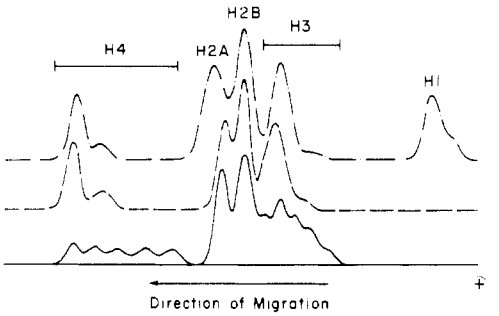

FIGURE 4: Electrophoretogram of histones from control and butyrate-treated high molecular weight chromatin stripped of H1 from HeLa cells. Scans of acetic acid–urea–13% polyacrylamide gels. (– –) Control H1-stripped chromatin; (—) H1-stripped chromatin from butyrate-treated cells; (–·–) control chromatin.

Table III: Transition Midpoints[a] and Relative Areas of Thermal Transitions[b] of Control and Acetylated, H1-Stripped Chromatin

	transition I		transition II		transition III	
	T_m (°C)	% H_T	T_m (°C)	% H_T	T_m (°C)	% H_T
control[c]	52	48	65	14	77	38
acetylated[c]	52	48	65	16	76	36

[a] T_m, temperature of maximum dh/dT for each transition, reproducible to ±2 °C. [b] % H_T, percent of total hyperchromicity found in a given transition, reproducible to ±5%. [c] All chromatin solutions are in 0.2 mM EDTA, pH 7.4.

T_m of acetylated chromatin is 4 °C lower than that of the control material. Thus despite the larger amount of material in transition III_A, the T_m is slightly lower. Interestingly, the values for T_m and % H_T of acetylated chromatin at 0 mM sodium phosphate are very similar to the values of control chromatin at 5.0 mM sodium phosphate. Thus acetylated chromatin is a more highly stabilized species, compared to control chromatin, and probably represents a more condensed state.

High Molecular Weight Chromatin Stripped of Histone H1. High molecular weight control and acetylated chromatin were stripped of H1 by passing high molecular weight chromatin obtained as described above through a Bio-Gel A-0.5m column equilibrated with 0.35 M NaCl, 10 mM Tris, and 0.25 mM EDTA, pH 7.4. Polyacrylamide tube gel electrophoresis of both the control and acetylated chromatin, so treated, revealed no H1 present, and most of the small amount of nonhistone chromosomal protein component previously shown to be present was removed (data not shown). Gel scans of the histone region (Figure 4) indicated that the core histones are still present in their original ratios, with no H1, and the chromatin from butyrate-treated cells is still highly acetylated.

The circular dichroism spectra of control and highly acetylated, high molecular weight, H1-stripped chromatin in 0.2 mM EDTA, pH 7.4, were the same, within experimental error. The $[\theta]_{282.5}$ was 5200 deg cm^2 (dmol)$^{-1}$ for stripped control chromatin and 5100 deg cm^2 (dmol)$^{-1}$ for stripped chromatin isolated from butyrate-treated cells, compared to 4900 deg cm^2 (dmol)$^{-1}$ for intact control chromatin. In the case of the stripped acetylated chromatin, the ellipticity at 282.5 nm represents an increase of 1400 deg cm^2 (dmol)$^{-1}$ with the removal of H1, compared with intact high molecular weight acetylated chromatin at the same ionic strength.

Thermal denaturation profiles were obtained for control and highly acetylated, H1-stripped chromatin at 0.2 mM EDTA, pH 7.4. The curves were resolved as described above, and the results are summarized in Table III. No difference can be detected between control and acetylated chromatin after re-

moval of H1. In comparing profiles from intact control (Table II) and acetylated, H1-stripped chromatin (Table III), it is observed that H1 removal does not affect melting profiles obtained from control chromatin but profoundly affects acetylated chromatin. For control chromatin with and without H1, transition I_C contains 48% of the hyperchromicity, transition II_C containing 14% and transition III_C containing 38% of the total hyperchromicity. In acetylated chromatin, transition I_A increases 24% in hyperchromicity with H1 removal, and transition III_A decreases 21%. The T_m of both transitions II and III is constant with H1 removal. Transition I in both control and acetylated chromatin shows a 3 °C decrease with removal of H1 and nonhistones. However, the decrease is within experimental error so that it may not be significant.

Discussion

A number of recent studies have indicated a relationship between histone acetylation and gene activity [for review see Allfrey (1980)]; however, the structural basis for this relationship remains unresolved. A variety of attempts have been made to obtain chromatin with modified histones and to correlate these modifications to changes in structure. One such modification, histone acetylation induced by sodium butyrate, has been shown to be associated with transcriptionally active chromatin as prepared by digestion with DNase I (Davie & Candido, 1980), DNase II (Davie & Candido, 1978), and micrococcal nuclease (Levy-Wilson et al., 1979).

DNase I has been used extensively to probe the structure of active chromatin (Gazit & Cedar, 1980). In addition, this enzyme has been shown to preferentially digest chromatin containing hyperacetylated histones as a result of sodium butyrate treatment in HTC cells (Sealy & Chalkley, 1978b; Nelson et al., 1978) and HeLa cells (Vidali et al., 1978a; Simpson, 1978). The results presented here are in agreement with these previous studies. The digestion of nuclei prepared from sodium butyrate treated cells proceeded at a faster rate and greater extent than the digestion of control nuclei digested under identical conditions.

The increased DNase I sensitivity of transcriptionally active chromatin containing hyperacetylated histones suggests that this chromatin is in a more extended conformation than bulk chromatin. Recently, Perry & Chalkley (1981) have shown that actively transcribing chromatin, containing hyperacetylated histones, has an increased solubility at physiological ionic strength compared to control chromatin. This result is also suggestive of a more extended conformation. An alternate explanation is the well-known association of HMG-14 and HMG-17 with DNase I sensitive sites on chromatin. Upon removal of HMG-14 and HMG-17 the DNase I sensitivity is lost (Weisbrod et al., 1980; Gazit et al., 1980). Thus an increased DNase I digestion of acetylated chromatin need not be solely due to an extended conformation.

Whether chromatin containing extensively acetylated histones is indeed in a more extended form can potentially be evaluated by physical studies obtained from butyrate-treated cells. Differences in physical parameters such as circular dichroism and thermal denaturation were not evident in the early work of Simpson (1978) on control and butyrate-treated high molecular weight chromatin and core particles obtained from HeLa cells. Vidali et al. (1978a) also examined control and highly acetylated HeLa mononucleosomes and observed no differences in the circular dichroism spectra or thermal denaturation profiles. Preliminary data on mononucleosomes from this laboratory are in agreement with those of Simpson (1978) and Vidali et al. (1978a) indicating no differences caused by acetylation. Bode et al. (1980) have reported studies that indicate that the mononucleosome structure is altered by butyrate treatment. It should be noted, however, that the effect of acetylation on the structure of high molecular weight chromatin and on monomers may be quite different due to the possible contribution of localized segments of acetylated and nonacetylated histones to higher order structure.

The results of Simpson (1978) on the physical properties of high molecular weight chromatin were obtained at one specific condition, very low ionic strength (0.25 mM EDTA). There is evidence (Fulmer & Fasman, 1979b) that the CD and thermal denaturation properties of erythrocyte chromatin vary systematically with ionic strength in the range of 0.75–7.0 mM sodium phosphate, indicative of a conformational change around 1.0 mM sodium phosphate. In this study, the CD and thermal denaturation properties of control and extensively acetylated chromatin were examined as a function of ionic strength to reveal any possible differences in the conformation and stability of these two chromatin samples.

Decondensation of chromatin structure was not detected in extensively acetylated chromatin as compared to control as might be expected from other studies. The opposite result was found, namely, sodium butyrate treatment of HeLa cells produced a condensation of the chromatin isolated. The CD spectra of DNA are sensitive to both secondary and tertiary structure. Core histone–DNA complexes have been observed to undergo a change in CD spectra relative to free DNA due to an induction of a broad negative band centered at 275 nm (Cowman & Fasman, 1978, 1980). This band, which is similar to the CD spectra observed for ψ DNA (Jordan et al., 1972), is thought to arise from the condensation of DNA into an ordered tertiary structure with no change in the secondary structure of DNA. Maestre & Reich (1980) have suggested that the ψ-type CD spectrum is a scattering resonance phenomenon due to side by side packing of DNA molecules with a helical twist and that, since the helicity of the tertiary structure is responsible for the ψ-type spectrum, a random condensation of DNA will not give a ψ-type CD spectrum. The induction of the negative CD band causes a decrease in the observed ellipticity from that observed for naked DNA [9000 deg cm^2 (dmol)$^{-1}$ (Cowman & Fasman, 1978)] to that found for highly condensed chromatin [2500 deg cm^2 (dmol)$^{-1}$ (Lee et al., 1981)]. Upon increasing the ionic strength, both control and acetylated chromatin indicated some condensation as evidenced by a decrease in ellipticity $[\theta]_{282.5}$ of approximately 900 deg cm^2 (dmol)$^{-1}$ (Figure 2 and Table I).

Hence, the observed decrease in ellipticity with increasing ionic strength is dependent on the proportion of DNA condensed into some tertiary structure. This result correlates with the electron microscope studies (Thoma et al., 1979) and CD studies of erythrocyte chromatin (Fulmer & Fasman, 1979a,b) that show a tightening of chromatin structure upon increasing ionic strength. Spectra obtained for acetylated high molecular weight chromatin show a similar ionic strength dependence, thus demonstrating that the acetylation did not prevent condensation. However, when comparing chromatin from control and butyrate-treated cells at the same ionic strength, the acetylated chromatin is more condensed than the control ($[\theta]_{282.5}$ of 3700 deg cm^2 (dmol)$^{-1}$ vs. 4900 deg cm^2 (dmol)$^{-1}$, respectively, at 0.2 mM EDTA, pH 7.4). This suggests that acetylation of core histones, accompanied by other changes caused by butyrate treatment, favors condensation of the type seen for ψ DNA.

Thermal denaturation profiles of both control and acetylated chromatin may be resolved into three transitions as described for chicken erythrocyte chromatin (Fulmer & Fasman, 1979b;

Cowman & Fasman, 1980). As indicated here, transition I_C represents melting of DNA that is relatively unprotected by interaction with proteins. Approximately 50% (96 base pairs/200 base pair repeating unit) of the DNA is of this type in control high molecular weight chromatin in 0.2 mM EDTA, pH 7.4. An increase in ionic strength to 5.0 mM NaH_2PO_4 decreases the amount of chromatin in this transition to 22% and is generally indicative of a condensation of structure as would be predicted on the basis of circular dichroism analysis. The second transition, II, has been suggested as being due to the binding of 40 base pairs of DNA onto the core particle by H1 (Fulmer & Fasman, 1979a,b). In the studies presented here, transition II_C remains relatively constant over the ionic strength range tested (Table II). Finally, the highest temperature transition, III_C, represents the remaining DNA base pairs bound most tightly to the nucleosome core. In this study, 72–126 base pairs out of the 200 base pair mononucleosome length are attributed to this category as a function of ionic strength. These observations suggest that the structure of chromatin compacted by ionic strength effects results from condensation of the DNA onto the core. The effects of ionic strength on the melting profile of acetylated chromatin were less dramatic. A slight decrease in transition I_A (4%) was paralleled by slight increases in transition III_A. Transition II_A was much more variable.

Comparison of thermal denaturation profiles for control and acetylated chromatin suggests that acetylated chromatin has much more DNA tightly bound in the high-melting region at low ionic strength (transition III_A, 59%, vs. III_C, 36%) (Table II). It appears that acetylated chromatin at 0 mM NaH_2PO_4 is condensed to a similar extent as control chromatin at 5.0 mM NaH_2PO_4.

The absolute $[\theta]_{282.5}$ values of acetylated chromatin indicate a higher degree of condensation, as does the thermal melting data, than that found for control chromatin at the lowest ionic strength. On raising the ionic strength to 5.0 mM NaH_2PO_4, $[\theta]_{282.5}$ decreases approximately equally for both chromatins, although there is not a comparable change in the thermal denaturation profile of acetylated chromatin. Thus it appears that a further condensation as displayed by CD is not accompanied by an equal stabilization of the DNA for acetylated chromatin, which is seen for the control.

The results presented here are contradictory to those of Simpson (1978), who showed no differences in the CD or thermal denaturation properties of control and acetylated chromatin in 0.25 mM EDTA whereas marked differences are reported herein. Further, Simpson (1978) reported an ellipticity of 4000 deg cm^2 $(dmol)^{-1}$ for high molecular weight chromatin isolated from control HeLa cell nuclei. This value stands in contrast to the ellipticity reported here of 4900 deg cm^2 $(dmol)^{-1}$ at the same ionic strength (0.25 mM EDTA, pH 7.4). In part, this discrepancy may be explained by differences in the protein:DNA ratio of chromatin, which in this study was 1.3:1 compared to that reported by Simpson (1978) of 2.9:1. Additional proteins could have marked effects on the properties of chromatin. In addition, the chromatin in the study of Simpson was prepared by osmotic lysis and homogenization, methods which may shear chromatin to a significant extent (Noll et al., 1975). Our method includes micrococcal nuclease digestion, which circumvents shearing forces but may select for a specific subclass of whole chromatin. Support for this idea is the recent finding of Kitzis et al. (1980) that at early stages of digestion with micrococcal nuclease, "butyrate-treated" chromatin was digested more rapidly than control chromatin. At longer digestion times, modified chromatin was attacked to a lesser extent than control chromatin. Thus the chromatin studied herein is probably a subset of the more slowly digested chromatin.

In attempting to understand the butyrate-induced structural changes of chromatin, it is important to note that sodium butyrate itself has manifold effects on the nucleus as well as histone acetylation. Acetylation per se may cause chromatin condensation, as described herein, only when accompanied by other, less obvious, butyrate effects. For example, butyrate causes a selective inhibition of ^{32}P incorporation into H1 and H2A in cultured HeLa S3 cells (V. G. Allfrey, personal communication).

Acetylation can affect chromatin structure in two ways. The first is by changing the binding properties of the core histones to DNA, causing an altered structure of the acetylated core particles. The second effect may be a modification of the higher order structure mediated through H1 by the binding of nonhistone proteins or modification of internucleosomal interaction. In this study, high molecular weight control and highly acetylated chromatin were stripped of H1 by passing the materials through a Bio-Gel A-0.5m column, equilibrated with 0.35 M NaCl, 10 mM Tris, and 0.25 mM EDTA, pH 7.4 (see Materials and Methods). Gel electrophoresis indicated that all of the H1 (Figure 4) and most of the nonhistone proteins (not shown) were removed. Thermal denaturation analysis showed no observable difference in profiles of control and highly acetylated, H1-stripped chromatin (Table III). The CD spectra of control and highly acetylated, high molecular weight, H1-stripped chromatin in 0.2 mM EDTA, pH 7.4, were the same within experimental error, $[\theta]_{282.5}$ = 5200 deg cm^2 $(dmol)^{-1}$ for control and 5100 deg cm^2 $(dmol)^{-1}$ for highly acetylated chromatin. Thus, stripping control chromatin caused little change in $[\theta]_{282.5}$ (4900 deg cm^2 $(dmol)^{-1}$) while stripping acetylated chromatin caused an increase of 1400 deg cm^2 $(dmol)^{-1}$. These observations indicate that the change in conformation observed in chromatin from butyrate-treated cells is a result of a structure formed in the presence of H1 (and perhaps nonhistone proteins) and when H1 is removed, acetylation of the core histones does not affect the structure of chromatin. It is interesting to note that the CD and thermal denaturation properties of control, H1-stripped chromatin are nearly the same for control chromatin with H1 while the highly acetylated chromatin shows a large decondensation with H1 removal. Thus the control chromatin studied herein appears decondensed. No explanation is at present obvious for this fact.

The results presented here suggest that acetylation alone is not sufficient to alter chromatin structure in such a way as to allow increased transcription. Thus, three main explanations for the observed effects of butyrate treatment may be suggested. First, acetylation of the N-terminal fragments of histones may weaken interactions between neighboring nucleosomes, allowing other factors (H1, nonhistones) to influence internucleosomal interactions more strongly. Depending on the environment, nucleosomes may move closer or further away from each other, producing differential sensitivities of the spacers toward micrococcal nuclease digestion as demonstrated by Kitzis et al. (1980). Results from this laboratory, as mentioned above, indicate that butyrate treatment alters the effects of H1 in some manner since acetylated chromatin stripped of H1 and nonhistones has the same ellipticity ($[\theta]_{282.5}$) as control, H1-stripped chromatin, at low ionic strength (0.2 mM EDTA). Second, several recent reports (D'Anna et al., 1980a; Whitlock et al., 1980) suggest that the alteration of chromatin structure may be under multiple levels

of control. Butyrate treatment in these studies links phosphorylation of H1 and the core histones to acetylation of H3 and H4. Butyrate has been shown to inhibit phosphorylation of H1 (V. G. Allfrey, personal communication). Lower levels of H1 phosphorylation would be expected to strengthen the binding of this protein to DNA, thus increasing condensation since the binding of H1 is correlated to the condensed structure (Thoma et al., 1979; Cowman & Fasman, 1980). As has been demonstrated above, depletion of H1 from butyrate-treated chromatin returns ellipticity values to control levels, exactly as predicted if H1 serves a condensing function. Finally, butyrate treatment has been shown to stimulate the synthesis of two proteins: BEP and UP (D'Anna et al., 1980b). On the basis of its migration, amino acid composition, and phosphorylation pattern during the cell cycle, BEP was tentatively indentified with the specialized class of histones known as $H1^0$. Histone $H1^0$ has been implicated in shutting down transcription in chromatin (Marsh & Fitzgerald, 1973; Pehrson & Cole, 1980). The synthesis of BEP increased 4–5-fold with butyrate treatment while UP increased twice. While no alteration in H1 phosphorylation or changes in the amounts or modifications of nonhistones were evident in this study, such changes have not been ruled out and may be responsible, at least in part, for the changes reported here.

Clearly, the process of transcribing a specific gene is a complex event in the cell nucleus from a structural point of view. It does not appear that a simple cause and effect relationship can be drawn between highly acetylated histones, actively transcribed genes, and a more extended conformation in chromatin due to effects caused by butyrate treatment. It appears that the consequences of such treatment appear to be more complex than the simple inhibition of the histone deacetylase enzyme. Further support for this notion appears in the studies of Lilley & Berendt (1979) where it was demonstrated that under identical conditions acetylated histones do not affect endogenous RNA polymerase template activity compared to nonacetylated histones. Dobson & Ingram (1980) report a slight increase in template activity. Shewmaker & Wagner (1980) demonstrated that acetylation of histones does not affect histone–DNA interaction by binding control and highly acetylated histones to SV40 DNA. The CD, thermal denaturation, and sedimentation studies of control and acetylated chromatin of Simpson (1978) and Vidali et al. (1978a,b) also support the idea that acetylation does not affect core particle structure. Whole chromatin obtained by sodium butyrate treatment of HeLa cells is found to be condensed, relative to controls, so this condensation must be mediated through H1 (and/or nonhistone proteins) and higher order structures. The results presented in this paper indicate that acetylation alone is not responsible for a loosening of chromatin structure but in coordination with other factors actually leads to condensation of that structure. It should be pointed out that condensation does not necessarily suggest inhibition of RNA polymerase binding to local regions of chromatin. Weintraub (1980) has proposed a model of compound recognition sites in DNA for RNA polymerase binding, which requires condensation of chromatin to align these recognition sites. The various modifications of chromatin that accompany butyrate treatment, as outlined above, may be responsible for decreased stability of local regions allowing RNA polymerase binding. Alternatively, correct alignment of signal sequences for effective RNA polymerase binding may actually require condensation of large segments of DNA since adjacent TATA boxes, which are thought to be signals for RNA polymerase binding, are located at great distances on the DNA backbone (Benoist et al., 1980; Mathis & Chambon, 1981).

Added in Proof

A recent paper by Xue & Rao (1981) showed that sodium butyrate arrested HeLa cells in the early G_1 phase with the chromatin being in a condensed state. This is in agreement with the results reported in this paper.

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Changes in DNA Polymerases α, β, and γ during the Replicative Life Span of Cultured Human Fibroblasts†

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ABSTRACT: DNA polymerases from IMR-90 human diploid fibroblasts at various passage levels and from HeLa cells were purified and fractionated into α_1, α_2, α_3, β, and γ species and subspecies, and then the accuracy with which each one copied synthetic template-primers was measured in the presence of Mn^{2+} or Mg^{2+}. All activities from fibroblasts of later population doubling levels incorporated noncomplementary triphosphates more frequently than did the same polymerase type from earlier population doubling levels. HeLa polymerase activities copied several different templates in the presence of Mn^{2+} with greater fidelity than enzymes from fibroblasts of population doubling level 27 or greater. The total DNA polymerase activity extracted from IMR-90 cells decreased with increasing population doubling levels. The α-polymerase activity generally declined with increasing population doubling levels, while β-polymerase activity remained relatively constant, except at the very end of the cellular replicative life span. In addition, the amounts of α_2 and α_3 became progressively lower relative to α_1, and a new α-type polymerase activity, α_0, appeared upon diethylaminoethylcellulose chromatography. HeLa cells also contained three α species, though two of them eluted from diethylaminoethylcellulose at higher phosphate concentrations than α species from fibroblasts. Postconfluent IMR-90 cells of population doubling level 21 had a decreased level of α-polymerase relative to that recovered from rapidly growing cells. This polymerase activity had some chromatographic properties similar to enzyme from late-passage cells. In addition, the α-, β-, and γ-polymerases from these cells had decreased fidelities relative to those isolated from subconfluent cells.

Alterations in the fidelity of DNA polymerases are expected to engender profound biological consequences. For example, several T4 mutants exhibit a relationship between changes in DNA polymerase fidelity and an increased or decreased mutation frequency (Speyer et al., 1966; Hall & Lehman, 1968). These altered mutation frequencies are often due to abnormal ratios of DNA polymerase activity to editing exonuclease activity (Muzyczka et al., 1972), though at least one of the T4 mutant enzymes selects noncomplementary triphosphates more frequently than does wild-type enzyme (Gillin & Nossal,

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