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# Chemical Inducers of Differentiation Cause Conformational Changes in the Chromatin and Deoxyribonucleic Acid of Murine Erythroleukemia Cells†

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ABSTRACT: The chemical inducers of murine erythroleukemia cell differentiation, dimethyl sulfoxide, sodium butyrate, and ethionine, elicited conformational changes in the DNA and chromatin of treated cells. The chromatin from dimethyl sulfoxide treated and butyrate-treated cells exhibited circular dichroic spectra different from that of the noninduced control. The molar ellipticity $[\theta]_{282.5}$ in isotonic saline decreased from 4900 deg·cm$^2$·dmol$^{-1}$ for control chromatin to 3800 and 3600 deg·cm$^2$·dmol$^{-1}$ for dimethyl sulfoxide treated and butyrate-treated chromatin, respectively, while that from ethionine-treated chromatin remained virtually unchanged (5400 deg·cm$^2$·dmol$^{-1}$). Increasing the ionic strength to 2.5 or 5 M with NaCl resulted in a substantial, uniform, decrease in molar ellipticity. Thermal denaturation profiles of high molecular weight DNA prepared from each of the inducer-treated cells showed a pronounced hyperchromic shift but no change in $T_m$ when compared to control DNA. Circular dichroic spectra of the DNA indicated a decrease in ellipticity $[\theta]_{277}$ from 9600 deg·cm$^2$·dmol$^{-1}$ to 8900, 8300, and 8800 deg·cm$^2$·dmol$^{-1}$ for ethionine, dimethyl sulfoxide, and butyrate treated cells, respectively. Treatment of the DNA with 3 M NaCl canceled the UV and CD differences. These measurements indicate an increased stacking of bases or an increased compactness of the DNA from induced cells. Concomitant with specific modifications such as hypomethylation of DNA, the data can be interpreted in terms of conformational changes in chromatin resulting from core histone acetylation.

The processes that mediate differentiation of murine erythroleukemia cell(s) (MELC) involve biochemical mechanisms that are not fully understood. Certain postsynthetic modifications of the macromolecular constituents of chromatin have been implicated. Inducers of MELC differentiation such as dimethyl sulfoxide ($Me_2SO$), butyrate, and ethionine have been shown to cause DNA hypomethylation, and this has been correlated with an increase in the expression of the globin gene (Christman et al., 1977; Van Der Ploeg et al., 1980; Weintraub et al., 1981). The possible relevance of DNA methylation to gene expression has been extensively reviewed (Drahovsky & Boehm, 1980; Razin & Riggs, 1980; Doerfler, 1981). The mechanisms by which these inducers of differentiation may cause DNA hypomethylation, however, are not entirely clear. Ethionine is a well-documented DNA methylase inhibitor (Cox & Irving, 1977) that interferes, as well, with lysine methyltransferase catalyzed histone methylation (Baxter & Byvoet, 1974). $Me_2SO$ and butyrate, on the other hand, have not been

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linked directly with the methylation process. Butyrate is a known inhibitor of histone deacetylase (Candido et al., 1978; Vidali et al., 1978), and hyperacetylation has been shown to occur in MELC as a result of butyrate treatment (Riggs et al., 1977). Recently, however, this agent has also been implicated in altered phosphorylation and methylation patterns in histone and nonhistone chromosomal proteins (Boffa et al., 1981). $Me_2SO$ has not been shown to interfere directly with mechanisms for covalent modification of polymers, but it is known to alter membrane structures (Eisen et al., 1977; Tapiero et al. 1980; Hunt & Marshall, 1981) and has profound effects on cellular levels of adenosine cyclic 3′,5′-phosphate (cAMP) and guanosine cyclic 3′,5′-phosphate (cGMP) (Chapman, 1981), a phenomenon that may lead to an altered phosphorylation pattern of chromatin proteins at the very least.

In recent years, it has been argued that the induction of MELC differentiation may involve DNA single-strand scission (Terada et al., 1978; Scher & Friend, 1978). This has not been confirmed by us or others (Pantazis et al., 1981; Wintersberger & Mudrak, 1982; Reboulleau et al., 1983), and a likely alternative involves conformational changes of the chromatin that could be mediated by certain postsynthetic modifications of its constituent molecules. Recently, Reczek et al. (1982) have been able to show that butyrate-treated HeLa cells do exhibit certain changes in chromatin structure.

In order to understand the effects of chemical induction on the conformation of the chromatin and constituent DNA from MEL cells during differentiation, thermal denaturation and circular dichroism analyses were performed. The changes in DNA and chromatin structures observed in these experiments are discussed in terms of the contributions made by the postsynthetic modifications that are known to occur in the chromatin.

## Materials and Methods

*Cells.* Murine erythroleukemia cells, strain 745A, were obtained from Dr. Judith Christman (Mount Sinai Medical School, CUNY, New York, NY). The cells were grown in suspension in minimum Eagle's medium containing glutamine and nonessential amino acids (GIBCO) and supplemented with 10% newborn calf serum, 250 units/mL penicillin G, and 0.2 mg/mL streptomycin sulfate. The cells were grown for a period of 72–96 h to a density of $10^6$ cells/mL. Cells induced to differentiate were grown in the presence of either 200 mM $Me_2SO$, 2 mM butyrate, or 4 mM DL-ethionine. Evaluation of differentiation based on hemoglobin synthesis was carried out by benzidine staining according to the method of Orkin et al. (1975), and cell viability was determined by trypan blue exclusion.

*Preparation and Characterization of DNA.* High molecular weight DNA from (1–2) × $10^8$ inducer-treated or control cells was prepared according to the method of Gross-Bellard et al. (1973). All dialysis membranes (Spectrapor-1) used in the preparation were pretreated by boiling in 10 mM $NaHCO_3$–1 mM ethylenediaminetetraacetic acid (EDTA) for 30 min, then boiling in bidistilled water, and thoroughly rinsing in water before use.

The molecular weight of the DNA from each preparation was measured by viscometry at 20 °C with a Beckman low-shear viscometer according to the method of Zimm & Crothers (1962). The concentration range for these measurements was (2–40) × $10^{-4}$ g of DNA/dL × $10^{-4}$. Cylinder rotation was at a shear, $\langle S \rangle$, of 0.0029 dyn·cm$^{-2}$ or less.

*Estimation of 5-Methylcytosine ($m^5C$) and Total Base Composition of Murine Erythroleukemia Cell DNA.* Freshly prepared DNA was exhaustively dialyzed against distilled water and lyophilized. The resulting material was hydrolyzed in sealed tubes at 175 °C for 35 min with 88% formic acid (0.1 mL/mg of DNA). The components of the hydrolysate were separated by solvent partition chromatography on Whatman 1 filter paper sheets using 86% 1-butanol or 86% 1-butanol–14% 1 N $NH_4OH$ as the descending solvent (Shapiro & Chargaff, 1960). The confluent UV adsorption zones corresponding to cytosine and 5-methylcytosine were exhaustively extracted with water. The solution was evaporated to dryness and resolubilized in 0.1 mL of bidistilled water. The proportion of each constituent was analyzed with a Whatman 10/25 SCX partisil ion-exchange column on a Waters Associates high-pressure liquid chromatography (HPLC) instrument. Other HPLC analyses were also carried out on total hydrolysates after removal of HCOOH, under vacuum, and dissolving the residue in distilled water.

Total composition of the DNA relied on standard UV spectrophotometric analyses of A, G, $m^5C$, C, and T from filter paper chromatograms (Shapiro & Chargaff, 1960).

*Thermal Denaturation of DNA Preparations.* DNA from noninduced control cells and inducer-treated MELC were exhaustively dialyzed against SSC/10 (0.015 M NaCl–0.0015 M sodium citrate) and adjusted to an absorbance of 0.5 at 260 nm. Thermal denaturation was carried out on a Gilford Model 2600 spectrophotometer with thermal programmer and printer accessories. Thermal denaturation was programmed between 60 and 95 °C. The percent hyperchromicity was computed, neglecting the dependence of hypochromism on helix length, since high molecular weight DNA was used in these experiments. The transition midpoint ($T_m$) of the derivative profile is defined as the temperature at a maximum $dh/dT$ and was reproducible within 2.0 °C. The denaturation profiles reported are representative of two to three melting curves for each DNA.

*S1 Nuclease Assay.* The sensitivity to S1 nuclease of DNA from uninduced MEL cells and cells induced to differentiate with $Me_2SO$ was assessed by low-shear viscometry at 20 °C. Measurements were made in the presence or in the absence of 0.5 or 2 units of enzyme per mL of DNA solution (~0.7 μg of phosphorus/mL) in 0.015 M NaCl adjusted to 0.025 N sodium acetate–1 mM $ZnSO_4$, pH 5.1 (Vogt, 1973). The observed changes in viscosity with respect to time of exposure to enzyme were reproducible in two to three different experiments for each enzyme concentration.

*Preparation of Murine Erythroleukemia Cell Chromatin.* MELC from noninduced control cultures and inducer-treated cultures were harvested at a density of $10^6$ cells/mL by centrifugation at 1800*g* for 5 min. The cells were washed twice with phosphate-buffered saline (PBS) and resuspended in 10–20 volumes of TKM buffer [50 mM tris(hydroxymethyl)aminomethane, pH 7.9, 25 mM KCl, 2 mM $MgCl_2$, and 2 M sucrose], and nuclei were prepared according to Spelsberg et al. (1974). The nuclear pellet was washed twice with 10 volumes of 10 mM Tris–0.15 M NaCl, pH 8. Chromatin was prepared according to the method of Nicolini & Baserga (1975) with minor modifications. The washed nuclei were allowed to swell in cold distilled water for 1 h in an ice bath and disrupted by gentle homogenization with a Teflon pestle–glass homogenizer. The lysate was layered on 3 volumes of 1.7 M sucrose and centrifuged at 38 000 rpm for 80 min in a SW 50.1 Beckman rotor. The chromatin pellet was resuspended in water and dialyzed against 10 mM Tris, pH 8, adjusted to either 0.01, 2.5, or 5 M NaCl. The chromatin solution was centrifuged at 15 000 rpm for 30 min in a Sorvall RC-2B equipped with an SS-34 rotor, and the ab-

Table I: Differentiation and DNA Composition of MELC

| cell treatment | viable cells (%) | benzidine + cells (%) | 100[m$^5$C/(m$^5$C + C)] | G[a] | C | A | T | G/C | A/T |
|---|---|---|---|---|---|---|---|---|---|
| none | 90 | 3.2 ± 0.5 | 3.52 ± 0.17 | 20.3 | 19.7 | 29.2 | 30.8 | 1.03 | 0.95 |
| $Me_2SO$ (200 mM) | 95 | 55.5 ± 9.8 | 3.36 ± 0.23 | 20.3 | 20.2 | 30.6 | 28.9 | 1.00 | 1.06 |
| butyrate (2 mM) | 97 | 43.8 ± 6.3 | 3.14 ± 0.31 | 20.5 | 20.8 | 29.4 | 29.3 | 0.99 | 1.01 |
| DL-ethionine (4 mM) | 95 | 13.0 ± 2.4 | 3.08 ± 0.15 | 20.8 | 20.0 | 29.3 | 29.9 | 1.04 | 0.98 |

[a] Proportions in moles of nitrogenous constituent per 100 mol of P in hydrolysate, corrected to 100% recovery.

sorbance was adjusted to unity at 260 nm.

*Circular Dichroism.* CD was measured on a Cary 61 recording spectropolarimeter. All measurements were carried out at 21 °C under nitrogen in fused quartz cells with a 0.2- or 0.5-cm light path, and scans were performed in the wavelength range 200–320 nm. Instrument sensitivity was maintained at 0.02° for full-scale deflection. The mean ellipticity ($[\theta]$) is expressed in degrees square centimeter per decimole of nucleotide residues, assuming an average molecular weight of 330 for nucleotides. DNA concentration was determined by the absorbance at 260 nm where a $A_{260}^{1cm}$ value of 20 = 1 mg/mL DNA. Reported spectra were the average of two to three scans obtained from two to three separate preparations of either high molecular weight DNA or MELC chromatin.

## Results

*Differentiation of Murine Erythroleukemia Cells.* The concentrations of chemical inducers were optimized to obtain maximum differentiation, as monitored by benzidine staining, without compromising cell viability. These data are indicated in Table I for cultures exposed for 72–96 h to inducing agents. Criteria for the termination of incubation depended on the time for cells to reach confluence [(0.6–1.2) × $10^6$ cells/mL] while maintaining maximum viability. $Me_2SO$- and butyrate-treated cells were usually slow to reach confluence (96 h of exposure as compared to 72 h for ethionine-treated or nontreated control cells), but they attained a higher degree of differentiation than ethionine-treated cells. In the case of butyrate-treated cells, confluence was reached at low cell density (0.6 × $10^6$ cells/mL), consistent with the known ability of butyrate to block DNA synthesis (Hagopian et al., 1977). The longer time required for $Me_2SO$-induced cells to reach confluence is more likely due to a lag observed in some instances after seeding in the presence of this inducer (Levy et al., 1975).

*Characterization of DNA from MELC.* DNA preparations obtained by the proteinase K procedure of Gross-Bellard et al. (1973) consistently yielded DNAs of molecular weights ranging from 0.6 × $10^8$ to 1.3 × $10^8$ as measured by low-shear viscometry. These measurements were done on at least three different preparations for each DNA from noninduced control and inducer-treated cells (data not shown).

The extent of hypomethylation was determined by HPLC, and total compositions were determined by spectrophotometric analyses after solvent partition chromatography. The results of these measurements are shown in Table I. The ethionine-treated cells showed significant hypomethylation. The other two cell treatments also afforded lower levels of 5-methylcytosine than that of the control, but statistical significance could not be rigorously demonstrated. The gross base compositions of the DNAs were identical for all cell treatments.

*Thermal Denaturation of DNA.* Thermal denaturation analyses were carried out on DNA preparations from inducer-treated and control MELC. The mean melting curves obtained are shown in Figure 1. There were no significant differences in the $T_m$ in SSC/10 observed for each DNA under our conditions of analysis: 71.5, 72.0, 71.0, and 72.0 for

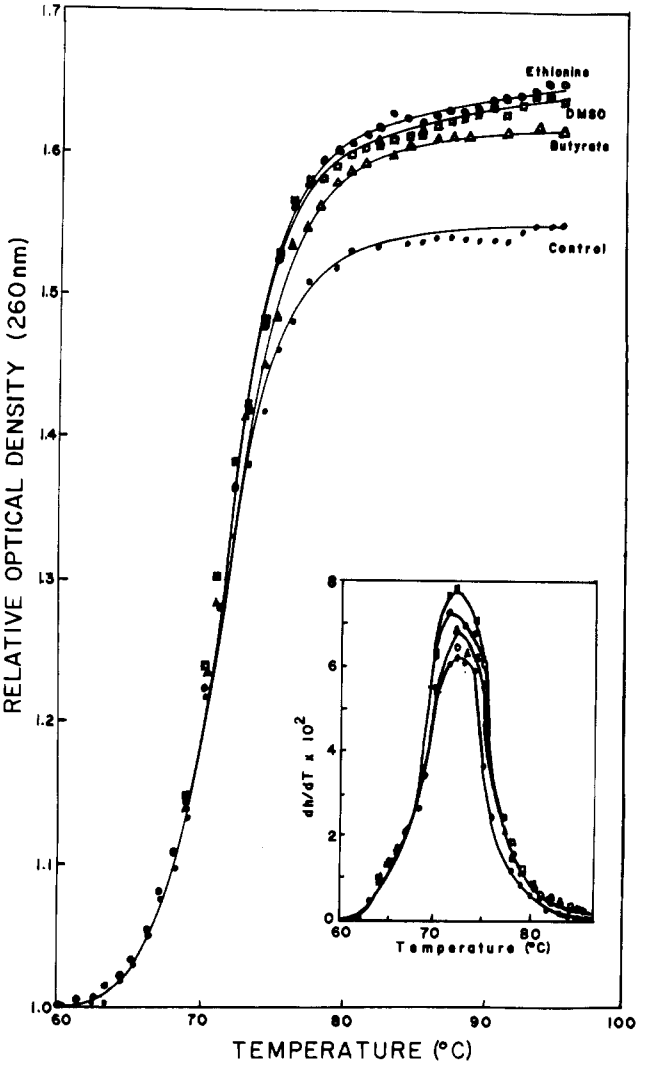


FIGURE 1: Thermal denaturation of DNA from inducer-treated and control cells. Inset: $dh/dT$ vs. $T$. Noninduced cells (●) and butyrate- (△), $Me_2SO$- (□), and ethionine-treated cells (○).

noninduced control, $Me_2SO$-treated, ethionine-treated, and butyrate-treated cell DNAs, respectively. The percent hyperchromicity was, however, higher for DNA from inducer-treated cells: 45.4%, 45.1%, and 48.0% for butyrate-, $Me_2SO$-, and ethionine-treated cells, respectively, compared to 40.9% for DNA from control, uninduced cells. These values were reproducible to ±2%. Establishment of the thermal denaturation profiles in the presence of 3 M NaCl abolished these differences, all, now, coincident to control (data not shown).

*Single-Strand Regions of DNA.* The extent of single-strand areas in the DNA from uninduced MEL cells and cells induced to differentiate by $Me_2SO$ was examined by low-shear viscometry at 20 °C. The sensitivity to S1 nuclease and the subsequent decrease in viscosity were used as criteria. Measurements were made with and without the addition of enzyme in 0.015 N NaCl. The viscosity changes recorded over a period

Table II: Summary of Data from Circular Dichroism Studies

| | cell treatment | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | control (no inducer) | | DL-ethionine (4 mM) | | $Me_2SO$ (200 mM) | | butyrate (2 mM) | |
| sample | $[\theta]_{277}$ [a] | crossover [b] | $[\theta]_{277}$ | crossover | $[\theta]_{277}$ | crossover | $[\theta]_{277}$ | crossover |
| DNA in | | | | | | | | |
| SSC/10 | 9600 | 257 | 8900 | 257 | 8300 | 257 | 8000 | 258 |
| 3 M NaCl | 4300 | 260 | 4000 | 260 | 4300 | 260 | 4400 | 261 |

| | control (no inducer) | | | DL-ethionine (4 mM) | | | $Me_2SO$ (200 mM) | | | butyrate (2 mM) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| sample | $[\theta]_{272.5}$ | $[\theta]_{282.5}$ | crossover | $[\theta]_{272.5}$ | $[\theta]_{282.5}$ | crossover | $[\theta]_{272.5}$ | $[\theta]_{282.5}$ | crossover | $[\theta]_{272.5}$ | $[\theta]_{282.5}$ | crossover |
| chromatin in | | | | | | | | | | | | |
| SSC/10 | 6000 | 4900 | 255 | 6700 | 5400 | 253 | 5500 | 3800 | 255 | 4000 | 3600 | 257 |
| 2.5 M NaCl | 2600 | 3300 | 260 | 3300 | 3700 | 258 | 3900 | 3600 | 256 | 3900 | 3300 | 257 |
| 5 M NaCl | 2100 | 2200 | 260 | 1500 | 2200 | 259 | 2500 | 2200 | 257 | 2300 | 2300 | 258 |

[a] Molar ellipticities are expressed as degrees square centimeter per decimole ± 300. [b] Crossover values are in nanometers.

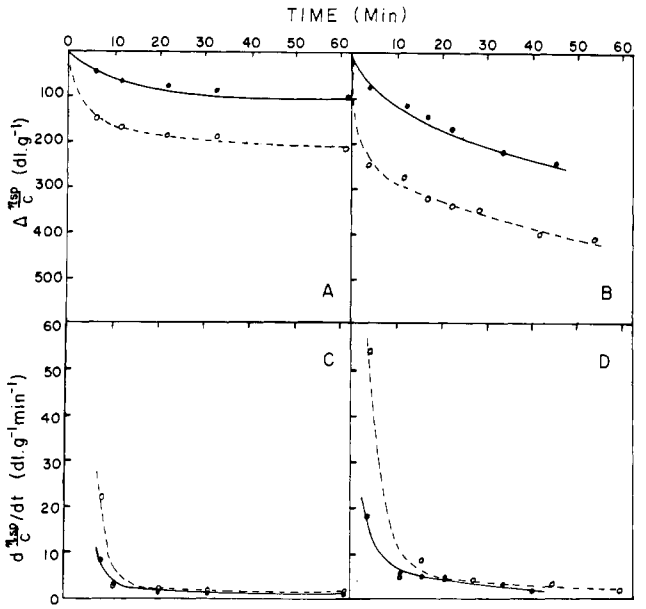


FIGURE 2: S1 nuclease dependent change in viscosity of DNA from control cells (—) and $Me_2SO$-treated cells (- - -). (A and C) $\eta_{sp}/C$ = 346 and 503 dL·g$^{-1}$ for control cells and $Me_2SO$-treated cells, respectively, before addition of S1 nuclease to a final concentration of 0.5 unit/mL. (B and D) $\eta_{sp}/C$ = 727 and 793 dL·g$^{-1}$ for control cells and $Me_2SO$-treated cells, respectively, before addition of S1 nuclease to a final concentration of 2 units/mL of DNA solution.

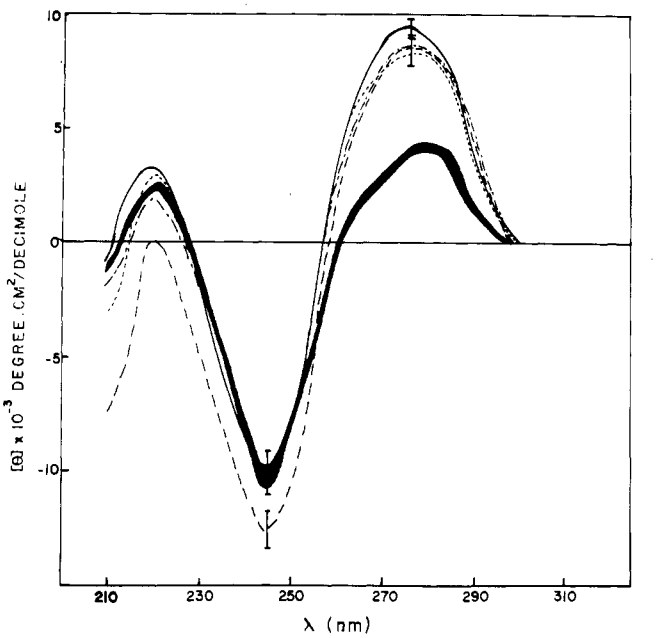


FIGURE 3: Circular dichroism spectra of high molecular weight DNA isolated from control (—), $Me_2SO$-treated (···), butyrate-treated (- - -), and ethionine-treated cells (-·-) in SSC/10. The superimposed spectra of the DNA in 3 M NaCl are represented by the solid ribbon.

of 60 min were calculated and plotted as $\Delta\eta_{sp}/C$ vs. time and $d(\eta_{sp}/C)/dt$ vs. time (Figure 2). The data clearly indicate a larger decrease in viscosity and a faster rate of decrease in the early stages of enzymic digestion for the inducer-treated cell DNA, while the rate of digestion is virtually the same for both DNAs in the late stages of the experiment.

*Circular Dichroism Measurements of DNA.* The circular dichroic measurements of the DNAs prepared from inducer-treated cells showed decreases in molar ellipticity at 277 nm. While there were no significant differences in positive molar ellipticity among inducer-treated cell DNAs (8900, 8300, and 8800 deg·cm$^2$·dmol$^{-1}$ for ethionine-, $Me_2SO$-, and butyrate-treated cell DNAs, respectively), these values were significantly different from that obtained for the DNA from noninduced control cells (9600 deg·cm$^2$·dmol$^{-1}$) (Figure 3). The CD profile of the DNA from butyrate-treated cells, moreover, exhibited a more pronounced negative ellipticity between 250 and 210 nm compared to all other cell treatments, including control. Measurements of circular dichroism in the presence of 3 M NaCl canceled these effects, and all four DNAs showed a positive ellipticity band between 4000 and 4500 deg·cm$^2$·dmol$^{-1}$ at 277 nm. All four CD profiles were, then, not significantly different from each other and are represented by the solid ribbon in Figure 3.

*Circular Dichroism of Chromatin.* A significant difference in positive molar ellipticity $[\theta]_{282.5}$ is observed between the spectra from $Me_2SO$- and butyrate-treated cell chromatins on the one hand (3800 and 3600 deg·cm$^2$·dmol$^{-1}$, respectively) and that from noninduced control and ethionine-treated cell chromatins on the other (4900 and 5400 deg·cm$^2$·dmol$^{-1}$, respectively). The CD spectra are shown in Figure 4. The protein area (240–200 nm) of the spectra is uniformly similar for all four cell types although an altered profile accompanied by a blue shift is distinguishable in the case of the ethionine-treated cell chromatin. Treatment of the chromatin preparations with 2.5 and 5 M NaCl resulted in the cancellation of these effects at 282.5 nm (Table II). This results in a net smaller decrease in positive ellipticity for $Me_2SO$- and butyrate-treated cell chromatin as compared to control chromatin and ethionine-treated cell chromatin. The data pertaining to the treatment of chromatin with NaCl are summarized in Table II.

## Discussion

We have used circular dichroism and thermal denaturation to examine the effects of the inducers of differentiation, bu-

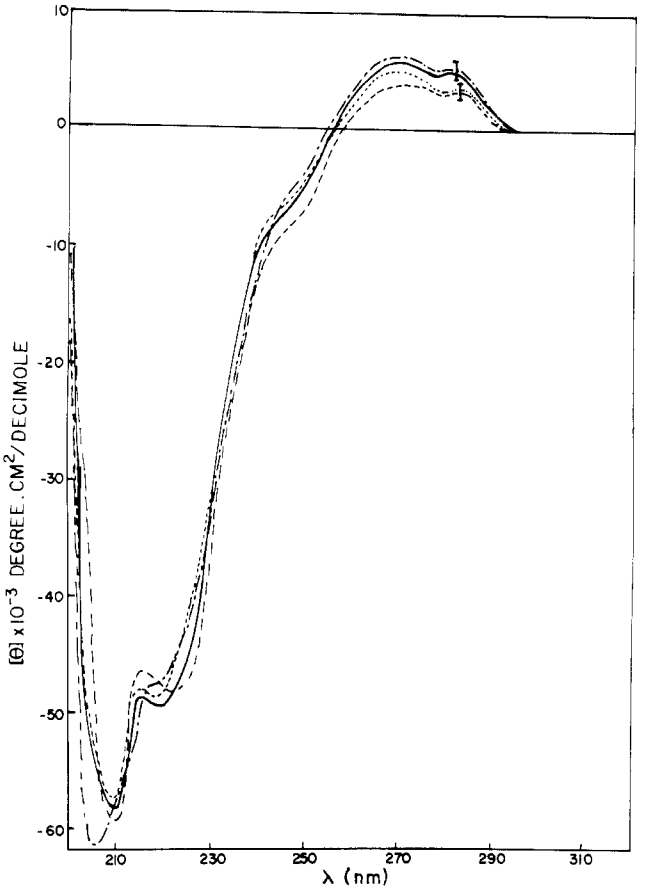


FIGURE 4: Circular dichroism spectra of chromatin isolated from noninduced control cells (—), $Me_2SO$-treated cells (···), butyrate-treated cells (- - -), and ethionine-treated cells (-·-).

tyrate, $Me_2SO$, and ethionine, on the conformation of the DNA and chromatin of MEL cells. Such chemical inducers alter the pattern of postsynthetic modification of the DNA and chromosomal proteins. Ethionine inhibits DNA methylation (Cox & Irving, 1977) and histone methylation (Baxter & Byvoet, 1974) while butyrate has been shown to be responsible for the alteration of phosphorylation, methylation, and acetylation patterns of chromatin proteins (Riggs et al., 1977; Candido et al., 1978; Vidali et al., 1978; Boffa et al., 1981). Such alterations have profound effects on the structure of the chromatin (Kitzis et al., 1980; Reczek et al., 1982). $Me_2SO$ is known to favor extensive rearrangements of membrane proteins (Tapiero et al., 1980) and to alter cyclic nucleotide levels (Chapman, 1981) with the possible result that different protein kinases are modulated by its action.

It has been possible to measure a decrease in positive ellipticity at 277 nm in the CD spectra of the DNAs from all three inducer-treated cell samples, indicative of a more condensed structure. Similarly, the DNAs isolated from inducer-treated cells all exhibit higher hyperchromicity than their nontreated counterpart, a feature that has long been associated with increased stacking of the bases (Tinoco, 1960; Rhodes, 1961).

While it can be shown that the induction of gene expression with butyrate, $Me_2SO$, or ethionine is correlated with significant hypomethylation of the DNA (Christman et al., 1977, 1980), the relation between hypomethylation and tertiary structure is not obvious. Highly methylated synthetic or natural DNA polymers have been shown to exhibit higher $T_m$ values than their unmethylated counterpart (Gill et al., 1974; Dawid et al., 1970). In this study, the level of hypomethylation is such that a difference in $T_m$ is not detectable. However, the hyperchromic shifts observed clearly indicate conformational rearrangements involving a generalized increase in base stacking and H-bond stability. Such changes may be due to a localized, selective transition between Z-DNA and B-DNA conformation (Wang et al., 1979) as Z-DNA conformation has been shown to be favored by the methylation of CpG repeat sequences (Behe & Felsenfeld, 1981). Simultaneously, while DNA may adopt a more condensed structure during specific gene expression, the S1 nuclease experiment indicates that concomitant with these changes, an increase in single-stranded areas occurs in the DNA of inducer-treated cells. It has been suggested that such features, in the form of cruciform loops, may play a role in the initiation of RNA synthesis (McKnight & Kingsbury, 1982).

The CD data obtained from chromatin samples were different from those obtained from purified DNA samples in several respects, indicating that chromosomal proteins play a significant part in maintaining or inducing rearrangements of DNA conformation within the chromatin. There were fewer differences in the positive ellipticities of the chromatin from noninduced and ethionine-treated cells, while the chromatin from $Me_2SO$- or butyrate-treated cells showed a significant decrease at 282.5 nm. This finding is consistent with the observation by Baserga et al. (1975) that WI 38 cells stimulated to proliferate have a chromatin exhibiting a higher positive ellipticity than quiescent cells. $Me_2SO$- and butyrate-treated cells are usually approaching terminal differentiation and cease dividing soon after complete induction of differentiation is attained (Friedman & Schildkraut, 1977). Ethionine, on the other hand, is a poor inducing agent, and the cells usually continue to divide in the presence of this inducer (Christman et al., 1977).

The positive ellipticity at 272.5 nm observed in these samples is high. Several factors may be responsible for this. All attempts were made to avoid shearing of the chromatin, but the method we have chosen to employ is less gentle than other methods relying on enzymatic digestion (Noll et al., 1975). The type of chromatin obtained in each case may be different since certain subclasses of chromatin are selected upon enzymatic digestions (Kitzis et al., 1980). Another consideration is that the cells used in our experiment are virally transformed. Huang & Baserga (1978) have reported higher positive ellipticities for the chromatin from SV 40 transformed WI 38 cells compared to their nontransformed counterpart. It is therefore possible that contribution from viral genomes may alter CD profiles. For simplicity, we have limited our discussion to the 282.5-nm band, a common practice.

The main difference in the CD profile at 282.5 nm is a less positive ellipticity at low ionic strength for the chromatin of $Me_2SO$- and butyrate-treated cells: 3800 and 3600 deg·cm$^2$·dmol$^{-1}$, respectively, compared to 4900 deg·cm$^2$·dmol$^{-1}$ for the nontreated control. Such a decrease has been observed in butyrate-treated HeLa cells and is explained in terms of the hyperacetylated state of the chromatin. It has been argued that acetylation of core histones along with other changes caused by butyrate treatment favors a condensation of the type seen for $\psi$ DNA (Reczek et al., 1982). Weintraub (1980) has proposed a model where certain DNA recognition sites involved in RNA polymerase binding require condensation of the DNA for alignment.

Hypomethylation, alone, whether of histones or of DNA, does not seem to cause measurable effects on chromatin conformation. The chromatin isolated from ethionine-treated cells does not present a CD spectrum significantly different from that of the control, with the possible exception of a blue

shift in the negative ellipticity protein band between 205 and 250 nm.

The involvement of DNA itself in the tertiary structure of the chromatin is demonstrated by the ineffectiveness of hyperacetylated histones to prevent further conformation condensation at higher ionic strength. The further decrease in positive ellipticity at 282.5 nm observed in the presence of 2.5 and 5 M NaCl indicates that this decreased ellipticity is dependent upon a certain proportion of DNA condensed into some tertiary structures, presumably the $\psi$ DNA structure. Finally, Reczek et al. (1982) have shown the importance of histone H1 in the condensation process as the stripping of H1 from the chromatin of butyrate-treated cells causes the ellipticity to return to control levels. Butyrate has been shown to inhibit H1 phosphorylation in a concentration-dependent fashion (D'Anna et al., 1980; Boffa et al., 1981), and a dephosphorylated histone H1 protein would be expected to bind more tightly to DNA. It is therefore significant that butyrate and $Me_2SO$ both alter cyclic nucleotide levels in differentiating MELC (Chapman, 1981) since it is possible that variations in the concentration of cyclic nucleotides are involved in modulating H1 binding to DNA and eventually controlling the maintenance of the condensed structure of the chromatin generated through core histone acetylation.

The data presented in this paper suggest that the process of cell differentiation involves several types of postsynthetic modifications of primary structure involving histone acetylation, DNA and histone methylation, and cyclic nucleotide dependent phosphorylation. These complex, interrelated changes may act in a coordinated manner to bring about the individual conformational changes in chromatin required for differential transcription and cell identity.

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**Registry No.** $Me_2SO$, 67-68-5; butyric acid, 107-92-6; DL-ethionine, 67-21-0; 5-methylcytosine, 554-01-8.

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