Histone H1 in Nuclei of Butyrate-Treated Murine Lymphosarcoma Cells Has Increased Affinity for Heparin

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Abstract. Nuclei from butyrate-treated murine lymphosarcoma cells were incubated with different amounts of the polyanion heparin, which is known to interact predominantly with chromatin-associated histones. Unlike isolated histone H1, histone H1 in the nuclei of butyrate-treated cells was found to display an enhanced affinity for the binding to heparin as compared to histone H1 from control cells. Dephosphorylation of histone H1 as a result of butyrate treatment of the cells is discussed as a possible factor involved in the observed higher affinity of the protein for heparin.

Key words: Histone H1 — Dephosphorylation — Heparin — Butyrate — Lymphosarcoma cells

Introduction

In recent years it was shown that sodium n-butyrate has a wide variety of effects on cells: these include e.g., inhibition of ^{3}H-thymidine incorporation and cell proliferation, arrest of the cells in the early G_1 phase of the cell cycle (for a review, see Kruh 1982). At the molecular level butyrate induces hyperacetylation of core histones, i.e. H4, H3, H2A and H2B (Riggs et al. 1977), and inhibits phosphorylation of histone (s) H1 (or H1 and H2A) (Boffa et al. 1981). Butyrate also induces structural changes in chromatin as shown, e.g., by CD spectra and T_m (Reczek et al. 1982), and by an increase in accessibility of some of its segments to DNase I (Nelson et al. 1978, Perry and Chalkey 1981) and micrococcal nuclease (Nelson et al. 1978). Another approach to the analysis of structural and functional properties of eukaryotic genome in nuclei or isolated chromatin are intercalating dyes (Paoletti et al. 1977) or polyanions (Hildebrand and Tobey 1975; Hildebrand et al. 1978).

Results from many laboratories (e.g. Berlowitz et al. 1972; Hildebrand et al. 1977) have shown that polyanions are agents which interact predominantly with chromatin-associated histones. Using acid extraction (Štros et al. 1984) we have

analyzed the fractions of nuclear chromatin histones bound to different amounts of the polyanion heparin, and could show that histone H1 in nuclei of butyrate-treated murine lymphosarcoma cells displayed a higher affinity for binding to heparin than did histone H1 from untreated cells.

Materials and Methods

Cell cultivation

Tumorigenic murine lymphosarcoma (LS/BL) cells were originally isolated by Jurášková (1965) and their growth characteristics in culture were described by Hill et al. (1970). LS/BL cells were weekly passaged in the peritoneal cavity of C 57 BL mice. The cells were then introduced into a primary suspension culture (1×10^6 cells/ml) in modified Eagle's medium containing 1 mg/ml galactose and 0.55 mg/ml pyruvate instead of glucose and supplemented with 10 % inactivated bovine serum, 5 % methocel, 1×10^5 IU penicillin and 0.1 g streptomycin/litre. The cells were grown in 8 % CO_2 atmosphere at 37 °C and continuously stirred. After 16—17 h of growth, the medium was replaced by a fresh medium and the cells were then cultured for 24 h with or without 5 mmol/l sodium n-butyrate.

Incubation of nuclei with heparin

Nuclei (250 μg DNA/ml) were prepared as described by Hymer and Kuff (1964), suspended in 0.25 mol/l sucrose, 10 mmol/l Tris.HCl (pH 8.0), 5 mmol/l $CaCl_2$, 1 mmol/l PMSF and 5 mmol/l sodium n-butyrate (for butyrate-treated cells only), and incubated with heparin (25—200 μg/ml, United Pharmaceutical Works, Czechoslovakia) at 4 °C for 30 min. Histones not bound to heparin were then extracted twice with 0.25 mol/l HCl, removed by centrifugation ($2500 \times g$ for 15 min), the pellet washed in acetone, air dried, dissolved in SDS-electrophoretic sample buffer, and submitted to SDS-15 % polyacrylamide gel electrophoresis according to Laemmli (1970). The hyperacetylation of histone fractions was assessed in acetic-urea-13 % polyacrylamide tube gels according to Traub and Boeckmann (1978). The resolved proteins were visualized by Coomassie blue R 250 staining. Some of the stained gels were scanned on a Pye Unicam SP 1700 model recording spectrophotometer with a SP 1809 densitometer accessory.

Pulse labelling of the cells with (^{32}P)orthophosphate

LS/BL cells were pulse-labelled for 2 h with (^{32}P)orthophosphate (3 MBq/ml) after 24 h of growing with or without 5 mmol/l sodium n-butyrate. After cooling, the cells were removed by centrifugation ($800 \times g$ for 5 min), washed in saline, and the nuclei isolated. All solutions for the isolation of nuclei contained 1 mmol/l PMSF and 20 mmol/l $NaHSO_3$. Proteins were then precipitated from isolated nuclei with 25 % trichloroacetic acid, washed in acetone, dried in air and subjected to SDS-15 % polyacrylamide gel electrophoresis according to Laemmli (1970). The resolved proteins were visualized by Coomassie blue staining followed by autoradiography. The dried gel was placed in contact with Medix Rapid X-ray film (Fotochema, Czechoslovakia). The film was exposed for 4 days at −70 °C and developed in Kodak D 19. The autoradiographs were scanned for quantitation of (^{32}P)-activity in individual protein bands.

Results and Discussion

It is well established that polyanions, when added to nuclei, chromatin or free histones, form complexes with histones which lose their extractability in 0.25 mol/l H_2SO_4 or 0.25 mol/l HCl (Berlowitz et al. 1972; Hildebrand et al. 1977). Histones,

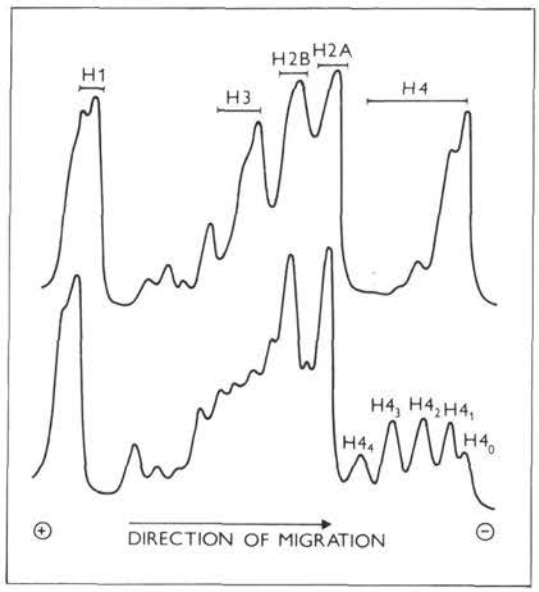

Fig. 1. Electropherogram of histones from control and butyrate-treated LS/BL cells. Scans of acetic acid-urea-13 % polyacrylamide tube gels (Traub and Boeckmann 1978). *Top*: control histones; *bottom*: histones from butyrate-treated cells.

free or bound in chromatin complex, interact with polyanions, including heparin, in the order of decreasing affinity:
H3 ~ H4 > H2B > H2A > H1 (Hildebrand et al. 1977; Štros et al. 1984).

We have studied the interaction of heparin with chromatin histones in nuclei from butyrate-treated LS/BL cells. Our analysis was restricted only to those proteins which appeared to be associated with heparin. The histones non-associated with heparin were removed by means of extraction with 0.25 mol/l HCl. The acid insolubility of the heparin-protein complexes (which was proved to be advantageous in the analysis) precluded, on the other hand, further analysis of heparin-associated histones according to the degree of their acetylation in acid-urea gel. For this reason, the histones together with the nonhistone proteins which were not acid-extractable (i.e. bound to heparin), were solubilized in 2 % SDS which was present in the electrophoresis buffer, and the dissolved proteins were submitted to SDS electrophoresis according to Laemmli (1970). The butyrate-induced hyperacetylation of histones H4 and H3 is shown in Fig. 1. The decrease of the positive charge of the core histones (i.e. H4, H3, H2A and H2B) caused by this hyperacetylation did not change the above-mentioned order of binding of histones to heparin (not shown). However, histone H1 in the nuclei of butyrate-treated LS/BL cells displayed an increased affinity for binding to heparin

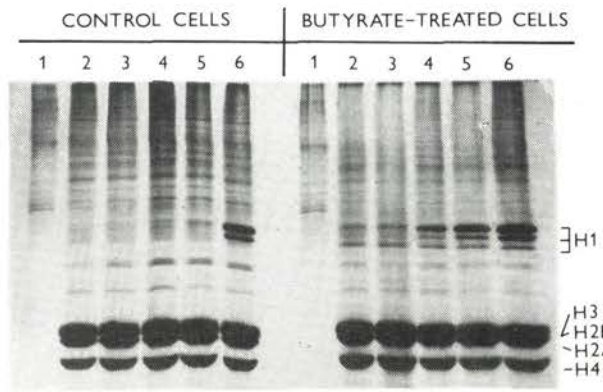

Fig. 2. Electrophoresis of nuclear proteins which are not extractable with 0.25 mol/l HCl after addition of heparin. Proteins were analyzed on SDS-15 % polyacrylamide slab gel (Laemmli 1970). The ratios of heparin/DNA (w/w) were: lane 1, 0; lane 2, 0.54; lane 3, 0.56; lane 4, 0.58; lane 5, 0.6; lane 6, 0.7. (A typical picture of 3 independent analyses.)

(Fig. 2). Histone H1 in the nuclei of control cells formed a complex with heparin (i.e., became unextractable with 0.25 mol/l HCl) at a ratio of heparin (DNA = 0.7, whereas histone H1 in the nuclei of butyrate-treated cells did so already at a ratio of heparin) DNA = 0.58. This finding could be explained either by an increased net positive charge of histone H1 as compared to histone H1 in control cells, or by an increased accessibility of histone H1 in the nuclei of butyrate-treated LS/BL cells to heparin, or by both.

Histone H1 or histones H1 and H2A have been found to be dephosphorylated in butyrate-treated CHO cells (D'Anna et al. 1980) or HeLa S3 cells (Boffa et al. 1981), respectively. As shown in our next experiment (Fig. 3), the treatment of LS/BL cells with 5 mmol/l sodium n-butyrate for 24 h resulted in a decrease of phosphorylation of histones H1 and H2A. Thus, hyperacetylation of core histones (Fig. 1) is linked with dephosphorylation of histones H1 and H2A (Fig. 3) in butyrate-treated LS/BL cells.

Dephosphorylation of histone H1 in butyrate-treated LS/BL cells may contribute to an enhanced affinity of histone H1 for heparin. However, the explanation of an enhanced affinity of dephosphorylated histone H1 in nuclei to heparin solely on the basis of the increased net positive charge of histone H1 is unlikely, since the precipitation curves of histone H1 isolated either from control or butyrate-treated cells with heparin are very similar (not shown).

Similary to other cell lines (Xue and Rao 1982; D'Anna et al. 1982; Darżynkiewicz et al. 1981), n-butyrate also blocks LS/BL cells in the early G_1 phase of the cell cycle (Široký and Štros 1983). A higher degree of chromatin condensation, as a result of butyrate treatment of the cells, was demonstrated by CD spectra (Reczek et al. 1982) and is in agreement with the arrest of the cells in

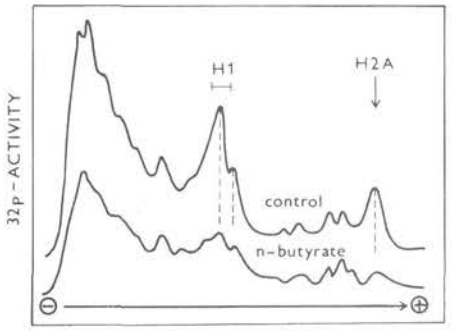

Fig. 3. Effect of sodium n-butyrate on phosphorylation of histones H1 and H2A of LS/BL cells. Comparison of (^{32}P)-activities of histones H1 and H2A of control and butyrate-treated cells. Nuclear proteins were analyzed by SDS-15 % polyacrylamide slab gel electrophoresis, visualized by Coomassie blue staining (not shown) followed by autoradiography, and scanned for quantitation of (^{32}P)-activity in individual protein bands. (Equal amounts of proteins in both lanes.)

the early G_1 phase of the cell cycle (Xue and Rao 1982; D'Anna et al. 1982; Darżynkiewicz et al. 1981). It was suggested by D'Anna et al. (1982) that dephosphorylation of the histone H1 would strengthen the binding of this protein to DNA, which would result in an increasing chromatin condensation since the binding of histone H1 is correlated to the condensed structure (Cowman and Fasman 1978). It is likely that dephosphorylation of histone H1 brings about structural changes in chromatin organization of the butyrate-G_1-arrested LS/BL cells, which may contribute to a higher accessibility of dephosphorylated histone H1 to heparin. However, it is not clear whether histone H1 in the chromatin from butyrate-treated cells is more exposed despite a higher degree of chromatin condensation. Recently, Doenecke (1982) has shown that chromatin subunits from butyrate-treated HeLa cells revealed an increased acidic extractability of histones H2A and H2B upon the addition of heparin. However, no decrease in the acid-extractability of histone H1 from isolated mononucleosomes was observed upon the addition of heparin. This negative finding could favour our suggestion that the influence of dephosphorylated histone H1 on the chromatin structure rather than dephosphorylation alone might explain the decrease in the acid-extractability of histone H1 in nuclei from butyrate-treated cells, since at the level of mononucleosomes, only an influence of dephosphorylation of histone H1 would be expected.

Hildebrand et al. (1978) have studied the interaction of heparin with intact nuclei isolated from CHO cells blocked in the early G_1 phase by isoleucine deprivation. They have found that phosphorylated histone H1 in nuclei displayed a decreased interaction with heparin compared to dephosphorylated histone H1. If we consider that CHO cells (D'Anna et al. 1982) and LS/BL cells in our

experiments (Široký and Štros 1983) are blocked both by isoleucine deprivation and 5 mmol/l sodium n-butyrate at about the same position of the G_1 phase (this does however not mean that the biological state of butyrate-blocked G_1 cells is the same as that of cells blocked by isoleucine deprivation), the results presented in this paper are comparable to and in agreement with the findings of Hildebrand et al. (1978) mentioned above.

In conclusion, heparin is an agent which can be employed for detecting changes in chromatin related to the cell cycle traverse as proposed earlier by Hildebrand and Tobey (1975). These changes probably correlate with histone H1 phosphorylation, which can contribute to the different affinity of the protein for heparin.

Abbreviations used: SDS — sodium dodecylsulphate
PMSF — phenylmethylsulphonylfluoride

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Received March 19, 1984/Accepted August 13, 1984