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THE PHOSPHORYLATION OF HIGH MOBILITY GROUP PROTEINS 14 AND 17 FROM EHRLICH ASCITES AND L1210 IN VITRO

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SUMMARY

The ability of the high mobility group (HMG) proteins to be phosphorylated was examined in Ehrlich ascites and L1210 cells incubated in vitro. HMG proteins were selectively extracted from isolated nuclei with 2% trichloroacetic acid, and electrophoretically separated on acid-urea or SDS polyacrylamide gels. Autoradiography of the gels revealed that among the HMG proteins, only HMG 14 and 17 were labeled. The specific activities of these two proteins were approximately equal to that of histone H1. Phosphorylation of HMG 14 and 17 reached a maximum in 2-3 hr and had turnover rates in pulse-chase experiments similar to that of phosphorylated histone H1.

INTRODUCTION

The high mobility group (HMG)[1] proteins are a class of chromosomal proteins operationally defined by their relatively high electrophoretic mobilities in acid-urea polyacrylamide gels (1). These proteins, extractable from chromatin with 0.35M NaCl and soluble in 2% TCA, have a large percentage of basic and acidic residues (1,2). The four major proteins of this group have been called HMG 1,2,14, and 17 (3), and have been isolated from a variety of species and tissues (4-6).

HMG proteins have been demonstrated to be associated with isolated nucleosomes (3,7,8), and those nucleosomes containing DNA enriched in transcribed sequences had increased amounts of HMG 14 and 17 (9). These two proteins may be responsible for sensitizing most genes to DNase I (10,11) which is believed to be a measure of potential gene activity (12).

[1]Abbreviations used: HMG, high mobility group; TCA, trichloroacetic acid; SDS, sodium dodecyl sulfate.

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It has been shown that HMG 1 and 2 and the erythrocyte specific HMG-E can be acetylated *in vitro* (13). This post-synthetic modification may have a role in chromatin structure and/or function. Many nuclear proteins, including the histones, also undergo phosphorylation. Sun *et al.* (14) have been able to thiophosphorylate calf thymus HMG 1 *in vitro* using a cAMP-dependent protein kinase. In this report, we have investigated the ability of the HMG proteins from Ehrlich ascites and L1210 cells to be phosphorylated. The results demonstrate that HMG 14 and 17, but not HMG 1 and 2, can be phosphorylated, and suggest that their function in transcription may depend on their behavior as phosphoproteins.

METHODS

Cell lines: Ehrlich ascites and L1210 cells were maintained in NIH Swiss and CDF_1 mice, respectively. Harvested cells were washed once with RPMI 1630 medium without phosphate, but supplemented with 20 mM HEPES (pH 7.4) and 10% fetal calf serum.

^{32}P labeling: Incubations of both cell lines were carried out for 3 hr at 37° in a shaking water bath. Each incubation consisted of 10^8 cells in 25 ml of modified RPMI 1630 as described above, with the addition of 2 mCi [^{32}P]phosphate.

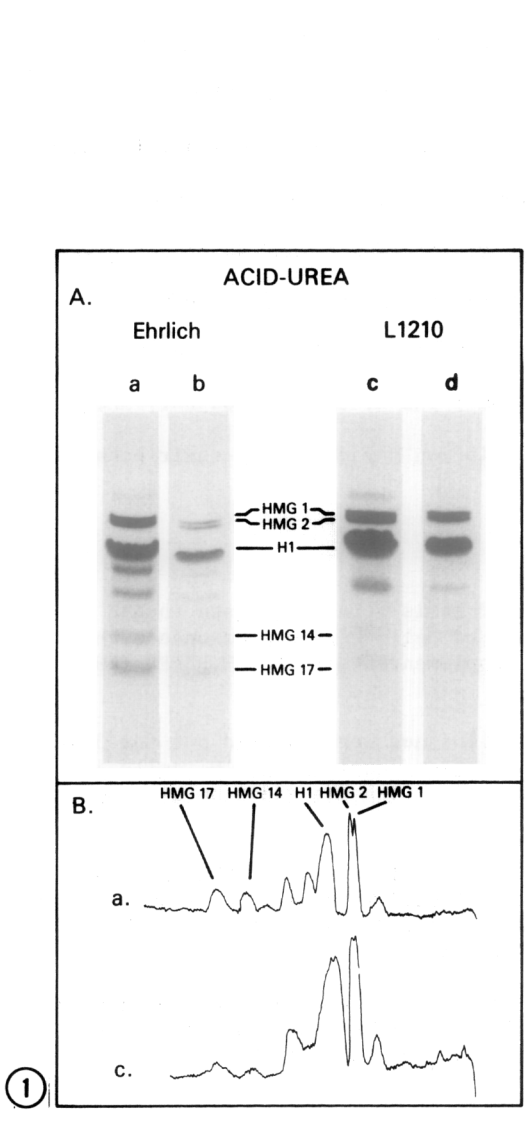
Isolation of HMG proteins: All buffers contained 0.5 mM phenylmethane sulfonyl fluoride added just prior to use. Nuclei were isolated from incubated cells by Triton X-100 lysis, followed by centrifugation through a 0.34M/0.88M sucrose step gradient containing 5 mM $MgCl_2$ (15).

The nuclear pellet was twice extracted with 2% TCA, and the combined extracts were precipitated with 25% TCA. The protein pellet was washed with cold acetone, dried, and the precipitated protein was dissolved in the appropriate gel buffer.

Electrophoresis: Electrophoresis in acid-urea gels was carried out by the method of Panyim and Chalkley (16). Electrophoresis in SDS gels of 16% acrylamide was according to the method of Laemmli (17). Proteins were stained with Coomassie Blue.

RESULTS

The 2% TCA extractable proteins from both Ehrlich ascites and L1210 cells were analyzed by acid-urea (Fig. 1) and SDS (Fig. 2) gel electrophoresis. These extracts contained HMG proteins 1,2,14 and 17, histone H1, and several nonhistone proteins. There are some notable differences between the extracts of the two cell lines. L1210 cells contain half the amount of HMG 14 and 17 of Ehrlich ascites cells. Ehrlich ascites cells also contain two additional



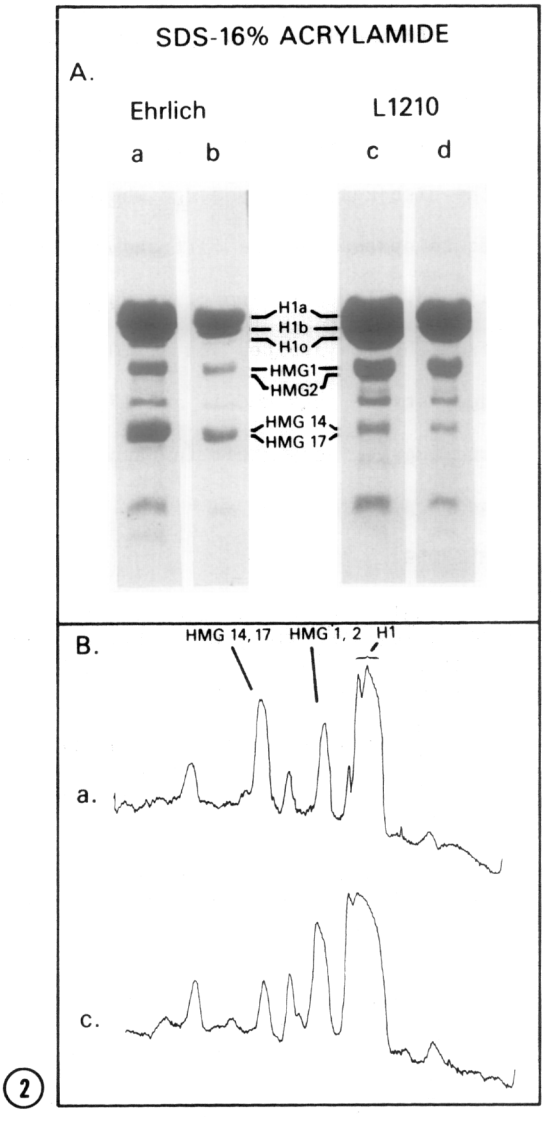


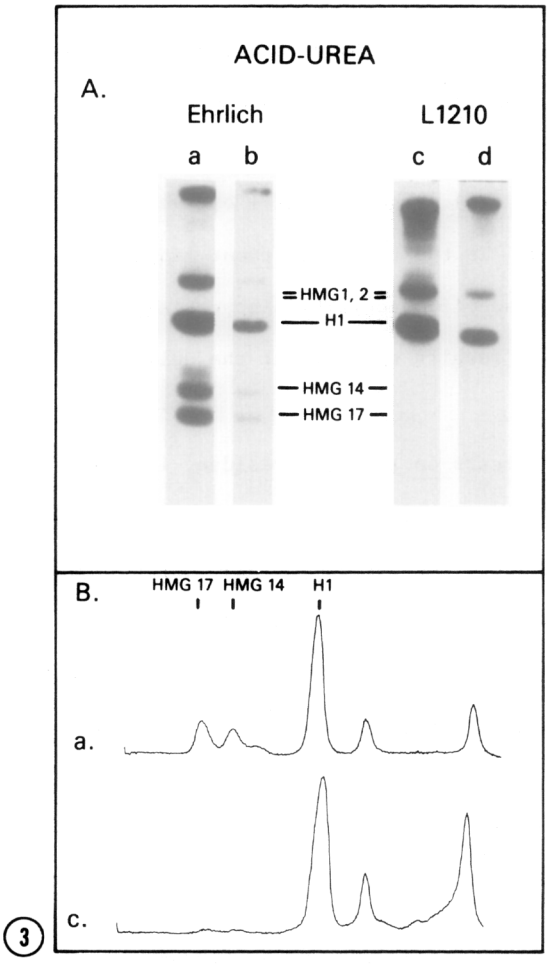
Figure 1. Acid-urea gels of 2% TCA extracts from Ehrlich ascites and L1210 nuclei. A. Electrophoresis of protein from each cell type. An amount of protein contained in approximately 2×10^7 cells was loaded in tracks a and c, and the protein in tracks b and d represents the amount contained in about 5×10^6 cells. B. Densitometer tracings of tracks a and c.

Figure 2. SDS gels of 2% TCA extracts from Ehrlich ascites and L1210 nuclei. A. Electrophoresis of proteins from each cell type. The two different amounts loaded are equal to those used in Figure 1. B. Densitometer tracings of tracks a and c.

proteins which migrate in acid-urea gels between histone H1 and HMG 14 (Fig.1a). The faster moving of these two non-HMG proteins comigrates with HMG 14 and 17 on SDS polyacrylamide gels (Fig.2a). The amounts of HMG 1,2,14, and 17 relative

to histone H1 from Ehrlich ascites cells are 0.35, 0.35, 0.20 and 0.25, respectively. The corresponding values for the L1210 proteins are very similar with the exception of HMG 14 and 17 as noted above.

Autoradiography of the gels of Figures 1 and 2 revealed that HMG 14 and 17 are labeled after incubating cells in the presence of [^{32}P]phosphate (Fig.3 and 4). These two proteins have specific activities approximately equal to that of histone H1. Apart from some minor differences between L1210 and Ehrlich ascites cells the major phosphoproteins were histone H1, HMG 14 and



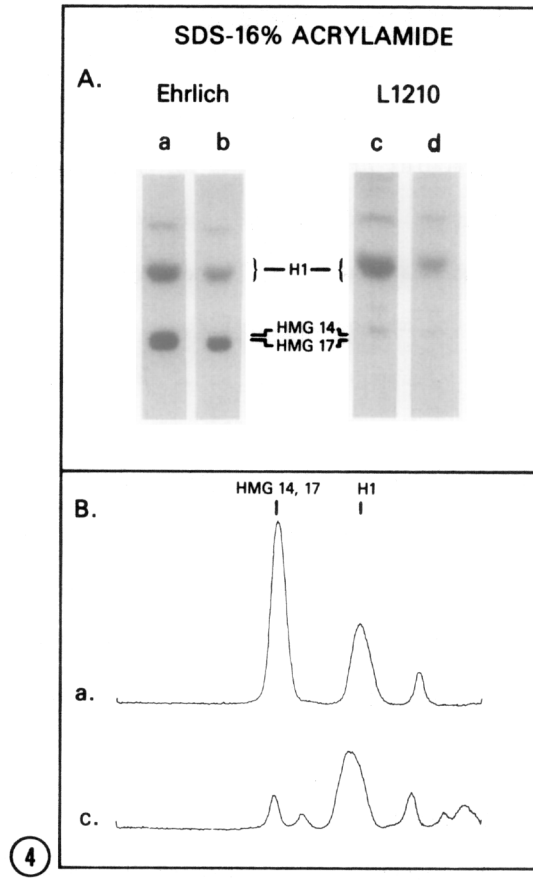


Figure 3. A. Autoradiographs of the acid-urea gels shown in Figure 1.
B. Densitometer tracings of tracks a and c.

Figure 4. A. Autoradiographs of the SDS gels shown in Figure 2.
B. Densitometer tracings of tracks a and c.

17, and a non-HMG protein migrating slower than histone H1 (Fig.3 and 4). The identification of [^{32}P]HMG 14 and [^{32}P]HMG 17 was confirmed by their elution from CM-Sephadex at pH 9 by 0.6M NaCl, and their pI = 6.5-7.5 upon isoelectric focusing. In addition, the degree of phosphorylation of all species reached a maximum in 2-3 hr, and the turnover rates of HMG 14 and 17 in pulse-chase experiments were similar to that of labeled histone H1.

DISCUSSION

The results presented demonstrate that HMG 14 and 17 are phosphorylated. In contrast, neither HMG 1 nor 2 are labeled during incubation of Ehrlich ascites or L1210 cells in the presence of [^{32}P]phosphate as might be suggested by the work of Sun et al. (14). It has been reported by Goodwin et al. (18) that HMG 1,2,3, and 17 from calf thymus are not highly phosphorylated. However, the data presented by Goodwin et al. (18) suggests that 1 of every 5 molecules of HMG 17 may be phosphorylated. In the two mouse tumor lines that we have examined, HMG 14 and 17 have specific activities approximately equal to that of H1. These differences in results may reflect the cells used or the method of isolation.

If phosphorylation of HMG 14 and 17 occurs on a serine residue, it may be in a sequence similar to -arg-arg-ser-ala-arg-leu-ser- which is conserved in calf thymus HMG 14 (19), HMG 17 (20), and trout testes H6 (21). Experiments determining the localization of HMG 14 and 17 in chromatin (3,8,22) and the role of these proteins in gene control (11) have not dealt with the possibilities of protein phosphorylation. The function of this protein modification may be to alter the interaction of the HMG proteins with DNA, histone, or other chromatin components and hence modulate transcription.

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