Volume 289, number 1, 113–116 FEBS 10111 September 1991

ADONIS 001457939100821F

Metaphase-specific* phosphorylations weaken the association between chromosomal proteins HMG 14 and 17, and DNA

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Received 17 June 1991

The high-mobility-group proteins HMG 14 and 17 have been isolated from human cells arrested in metaphase. The affinity between an unphosphorylated and two phosphorylated forms of these proteins, and DNA has been investigated using columns of single-stranded and double-stranded DNA. It was shown that the most phosphorylated forms had much lower affinity for single-stranded and double-stranded DNA compared to the unphosphorylated form present in interphase cells. The results are in accordance with the view that HMG 14 and 17 may dissociate transiently from chromatin during mitosis.

Metaphase; Phosphorylation; High-mobility-group proteins; DNA affinity.

1. INTRODUCTION

HMG 14 and 17 are two well-characterized chromosomal proteins which probably are preferentially associated with active genes and might have a function concerned with gene transcription [1–4]. These two proteins preferentially associate with single-stranded DNA [5]. Their function is probably highly regulated by phosphorylation, since both proteins are phosphorylated by protein kinase C, c-AMP dependent kinase and c-GMP dependent kinase in vitro and furthermore, HMG 14 is a substrate for casein kinase II [6–8]. The positions of the amino acids phosphorylated by protein kinase C seem to be in the region of HMG 14 and 17 which is associated with DNA [6,16].

We have shown that neither HMG 14 or 17 is labelled to any significant degree when HeLa cells are grown exponentially with a ^{32}P-containing medium [9], suggesting that they could be phosphorylated only transiently during the cell cycle. On the other hand, two different phosphorylated forms have been detected when HMG 14 and 17 were isolated from metaphase arrested cells [9,10].

In order to investigate the effect of these probably mitosis-specific phosphorylations, chromatography on columns containing immobilized double- and single-stranded DNA was undertaken, and it was found that phosphorylation highly decreased the affinity for DNA.

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2. MATERIALS AND METHODS

2.1. *Propagation of cells*

HeLa S3 cells were propagated in suspension culture at a density of 50 × 10^4/ml and arrested in metaphase with colcemid as in [9]. The established cell line NHIK 3025, derived from a carcinoma in situ of the cervix [11] was subcultured 2 times a week in Medium E2a [12], containing 20% human serum and 10% horse serum. Mitotic cells were obtained by incubating the cells with 1.0 μg/ml nocodazole (Aldrich Chem.) and shaking the cells in monolayer on a reciprocal shaker for 60 s. The mitotic index was higher than 90%.

2.2. *Extraction of HMG proteins, and purification of HMG 14 and 17*

HMG proteins were extracted from whole cells with 5% perchloric acid and precipitated with acetone/HCl. Precipitated proteins were fractionated by chromatography on phosphocellulose as in [9]. HMG 14- and 17-containing fractions were collected and these proteins were finally purified on HPLC using an acetonitrile gradient as described in [13].

2.3. *Chromatography on columns containing immobilised DNA*

A column of single-stranded DNA-agarose (Pharmacia) was equilibrated with 100 mM NaCl, 1 mM Tris-HCl, and proteins were eluted with a linear gradient of NaCl. A column of double-stranded DNA-cellulose (Pharmacia) was equilibrated with the same buffer and proteins were eluted with a linear gradient of NaCl.

2.4. *Polyacrylamide gel electrophoresis*

Acetic acid/urea gels were composed of a stacking gel containing potassium acetate (pH 4.0) and a resolving gel containing 15% acrylamide, 2.5 M urea and 0.9 M acetic acid as in [9].

3. RESULTS AND DISCUSSION

HMG proteins were extracted with 5% perchloric acid from HeLa S3-cells arrested in metaphase with colcemid. The soluble proteins were analysed by acetic acid/urea gel electrophoresis which separates the phosphorylated forms of the small HMG proteins [9]. Two different phosphorylated forms of HMG 14 and 17,

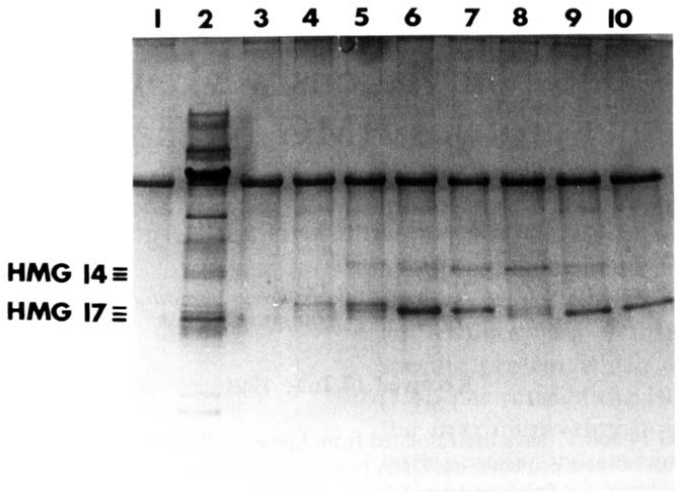

Fig. 1. Acetic acid/urea gel electrophoresis of the protein content of different fractions collected from chromatography on single-stranded DNA-agarose. Lane 1, histone H1 used as carrier; lane 2, perchloric acid soluble proteins from HeLa S3 cells arrested in metaphase; lane 3, the fraction eluted between 153.33 and 166.67 mM NaCl; lane 4, the fraction eluted between 166.67 and 180.0 mM NaCl; lane 5, the fraction eluted between 180.0 and 193.33 mM NaCl; lane 6, the fraction eluted between 199.33 and 206.67 mM NaCl; lane 7, the fraction eluted between 206.67 and 220.0 mM NaCl; lane 8, the fraction eluted between 220.0 and 233.33 mM NaCl; lane 9, the fraction eluted between 233.33 and 246.67 mM NaCl; lane 10, the fraction eluted between 246.67 and 260 mM.

probably mono- and diphosphorylated, have been identified earlier [9]. In Figs. 1 and 2 the two upper of the three labelled bands of HMG 14 and 17 represent the phosphorylated forms.

HMG 14 and 17 used in the subsequent chromatography on single-stranded and double-stranded DNA columns were purified by chromatography on phosphocellulose and HPLC. The binding to the DNA columns was therefore due to interactions between HMG 14 and 17 and DNA and not due to interactions between HMG 14 and 17 and other DNA-binding proteins.

A mixture of HMG 14 and 17 was applied to a single-stranded DNA-agarose column equilibrated with 100 mM NaCl, 1 mM Tris-HCl, pH 7.5 and eluted with a linear NaCl gradient. Fractions, each representing an increase in salt concentration of 13.33 mM, were precipitated with TCA using histone H1 as carrier, and then analysed by acetic acid/urea gel electrophoresis (Fig. 1). The forms of HMG 14 and 17 with the lowest mobility, which probably are the most phosphorylated forms, mainly eluted in the fraction between 180 and 193 mM NaCl (lane 5). Monophosphorylated HMG 17 eluted in the fractions between 193–206 mM and 206–220 mM

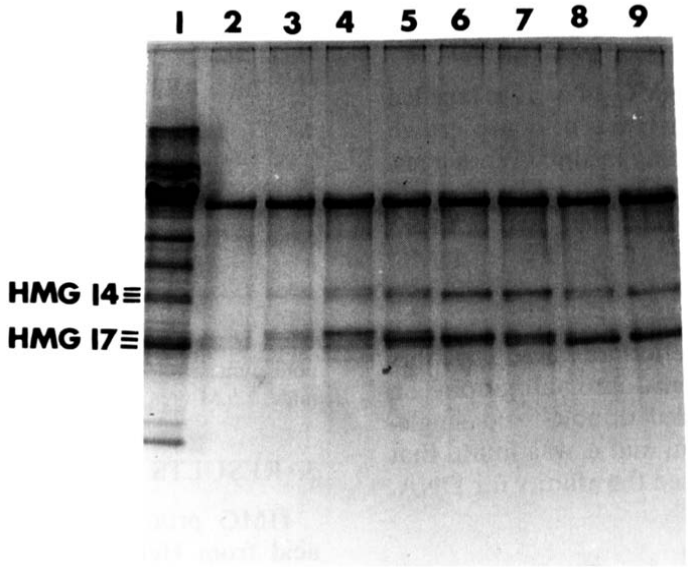

Fig. 2. Acetic acid/urea gel electrophoresis of the protein content of different fractions collected from chromatography on double-stranded DNA-cellulose. Lane 1, perchloric acid soluble proteins from HeLa S3 cells arrested in metaphase; lane 2, the fraction eluted between 160 and 170 mM NaCl; lane 3, the fraction between 170 and 180 mM NaCl; lane 4, the fraction between 180 and 190 mM NaCl; lane 5, the fraction between 190 and 200 mM NaCl; lane 6, the fraction between 200 and 210 mM NaCl; lane 7, the fraction between 210 and 220 mM NaCl; lane 8, the fraction between 220 and 230 mM NaCl; lane 9, the fraction between 230 and 240 mM NaCl.

(lanes 6 and 7), with the highest amount in the first fraction. Unphosphorylated HMG 17 eluted in two fractions between 233 and 260 mM NaCl (lanes 9 and 10). Monophosphorylated HMG 14 eluted mainly in the fraction between 220 and 233 mM NaCl. The sample used contained very small amounts of unphosphorylated HMG 14 and that form was not detected clearly.

The recorded absorbance at 214 nm revealed two peaks at respectively 200 mM and 242 mM NaCl indicating that monophosphorylated and unphosphorylated HMG 17 were eluted at those concentrations, while a shoulder at 186 mM probably contained diphosphorylated HMG 17 (not shown). It therefore seems to be that unphosphorylated HMG 17 binds to single-stranded DNA at a NaCl concentration which is 56 mM higher than that sufficient to dissociate the most phosphorylated form present in metaphase cells.

The binding between HMG 14 and 17 and double-stranded DNA was also investigated. HMG 14 and 17 isolated from metaphase-arrested cells were applied to a column of double-stranded DNA-cellulose equilibrated with 100 mM NaCl, 1 mM Tris-HCl, and eluted with a linear gradient of NaCl. Fractions at intervals of 10 mM NaCl were collected and protein precipitated with TCA using H1 as a carrier. The protein content in each fraction was analysed by acetic acid/urea gel electrophoresis (Fig. 2). The most phosphorylated forms of HMG 14 and 17 eluted mainly in the fraction between 180 and 190 mM NaCl (lane 4), although some HMG 17 eluted in the fraction between 170 and 180 mM NaCl (lane 3). The forms of HMG 14 and 17 which probably are monophosphorylated eluted mainly in the fraction between 200–210 mM NaCl (lane 6) while unphosphorylated HMG 14 and 17 eluted between 220 and 240 mM NaCl (lanes 8 and 9).

The absorbance at 214 nm recorded during the elution gave peaks at respectively 205 mM and 230 mM NaCl probably representing monophosphorylated and unphosphorylated HMG 17 and a shoulder at 185 mM probably being diphosphorylated HMG 17 (not shown).

In this work it is shown that the association between the most phosphorylated forms of HMG 14 and 17 present in metaphase cells and DNA is much weaker than the association between unphosphorylated HMG 14 and 17 and DNA. Especially the affinity for single-stranded DNA is much lower.

It has been suggested earlier that only a fraction of HMG 17 is associated with DNA in metaphase cells [10]. The results presented here could give an explanation for the presence of HMG 17 in the soluble fraction from metaphase cells. Diphosphorylated HMG 17 and 14 were dissociated from the DNA columns at a NaCl concentration which is only slightly higher than that present in the cell. Taken into account that different cations in addition to sodium are present in the cell and that other cellular proteins could compete with HMG

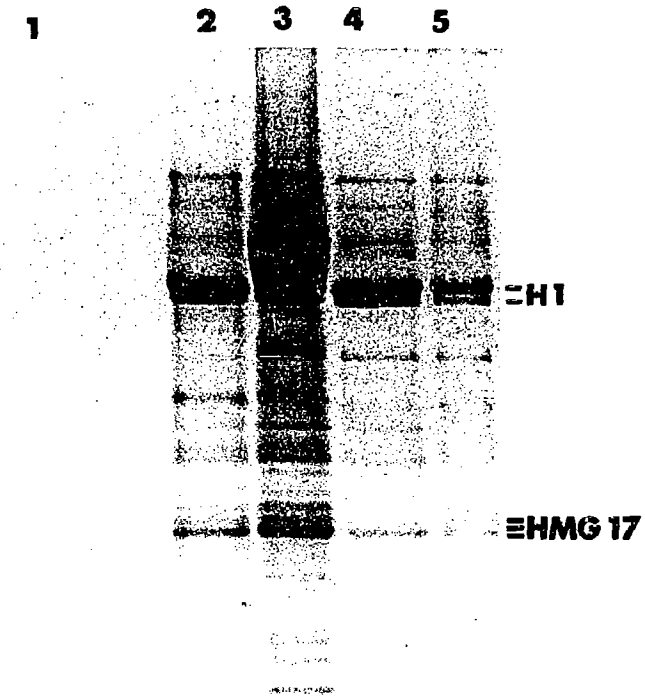

Fig. 3. Acetic acid/urea gel electrophoresis of perchloric acid soluble proteins isolated from cells collected at different intervals after nocodazole or colcemid addition. Lane 1, unphosphorylated HMG 17 isolated from pig thymus; lanes 2 and 3: perchloric acid soluble proteins from HeLa cells collected 24 h after colcemid addition; lane 4, perchloric acid soluble proteins from NHIK 3025 cells collected 24 h after nocodazole addition; lane 5, perchloric acid soluble proteins from NHIK cells collected 3 h after nocodazole addition.

17 for DNA sequences, it is not unreasonable that the metaphase-specific phosphorylations are sufficient to dissociate HMG 17 from DNA.

It has recently been suggested that chromosomal proteins, transiently released to the cytoplasma during mitosis, might preferentially reassociate with chromosome regions that contain DNA with special conformations and facilitate decondensation [14]. In accordance with this view our suggestion that HMG 14 and 17 might dissociate from chromatin transiently during mitosis is of interest, since these proteins probably are components of the open configuration of transcriptionally active chromatin [1–4].

The phosphorylated amino acid residues on HMG 17 are probably Ser^{24} and Ser^{28}, since human HMG 17 contains no other serine residues and only one threonine which is apart from the DNA binding domain [15]. Furthermore, it has been suggested that the DNA-binding region contains Ser^{24} and Ser^{28} [16]. It has previously been shown that HMG 17 is phosphorylated by protein kinase C in vitro at Ser^{24} and Ser^{28} [6]. This identity suggests that PKC might be responsible for the metaphase-specific phosphorylations of HMG 17 and that this enzyme also plays a regulatory role at mitosis.

It has been reported that the most phosphorylated form of HMG 17 constitute only about 6% of the total

amount of HMG 17 in metaphase cells [10]. To investigate whether the low amount of this fraction was due to dephosphorylation after some time in metaphase arrest, perchloric extracts from metaphase cells collected at different intervals after nocodazole addition were compared. The ratio between the different forms of HMG 17 was found to the about the same in the extract from NHIK 3025 cells harvested after 3 h with nocodazole as in the extract from cells harvested 24 h after this addition (Fig. 3). In contrast it could be seen that in the case of NHIK 3025 cells some histone H1 probably was dephosphorylated after 24 h in metaphase arrest. These results support the view that only a fraction of HMG 17 dissociates from DNA during metaphase.

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