Preferential Phosphorylation of High Mobility Group Protein 17 *in vitro* by a Nuclear Protein Kinase

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High Mobility Group Proteins, Phosphorylation, Nuclear and Cytoplasmic Kinase

The ability of the high mobility group proteins (HMG-1, 2, 14 and 17) to serve as substrate for protein kinases was investigated by incubating them with a cytoplasmic and nuclear kinase. In both cases phosphate was incorporated into all four HMG proteins. The amount of phosphate incorporated and the specificity for the four proteins was quite different for the two kinases. Whereas the cytoplasmic kinase phosphorylated the HMG-1 and 2 to a higher degree than HMG-14 and 17, the nuclear kinase exhibited a high specificity for the HMG-17, leaving the other three proteins with only a small amount. The high preference of a nuclear kinase for HMG-17 may be indicative of a specific phosphorylation occuring also *in vivo*.

Introduction

The high mobility group proteins (HMG-1, 2, 14 and 17) constitute a subclass of non-histone chromosomal proteins of obviously general occurence in the eucaryotic kingdom [1–4]. Their association with nucleosomes and with DNase I – sensitive regions of the genome [5–8] is indicative of the role played by these proteins in chromatin organization and probably also in the control of gene expression. These functional implications lead to the expectation that HMG proteins are subjected to similar post-synthetic modifications as it is known for the histones or other non-histone proteins. Very recently the post-synthetic acetylation [9] and methylation [10] of HMG-1 and HMG-2 and the phosphorylation of HMG-14 and HMG-17 was reported [11, 12]. However, until now there is no evidence of the enzymic phosphorylation of the two larger HMG proteins HMG-1 and HMG-2.

In order to evaluate whether the four HMG proteins are substrates of protein kinases, *in vitro* phosphorylation was performed with two different enzymes: a nuclear, cAMP-independent kinase from pig liver and a cytoplasmic, cAMP-dependent protein kinase from rabbit muscle (catalytic subunit). The choice of these two kinases may also be correlated with the question, whether a possible phosphorylation of these proteins *in vivo* occurs in the cytoplasm or in the nucleus.

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Materials and Methods

The HMG proteins were isolated from chicken erythrocyte nuclei as described in [13, 14] with slight modifications. The lysis buffer contained 5% Triton instead of saponin and the purification of the nuclei was performed in the presence of 0.02% NaN_3. The coextracted histone H1 was separated from the HMG proteins by precipitation with acetone. The HMG proteins were identified by acrylamide gel electrophoresis containing 0.1% sodium dodecylsulfate [15]. After staining with Coomassie Brillant Blue G-250 the gels were scanned at 580 nm and the relative amounts of the four proteins determined by weighing the areas under the respective peaks.

Isolation of the catalytic subunit of protein kinase I from rabbit skeletal muscle was performed according to [16]. The nuclear kinase from pig liver was prepared following a procedure described for a similar enzyme from rat liver nuclei [17] introducing, however, several modifications [18]. These include the use of 20 mM MES buffer of pH 6.9 and the application of Blue Dextran affinity chromatography, and result in a homogeneous and highly active enzyme [18]. The details of the procedure and the properties of the kinase are described in [19].

For phosphorylation the HMG proteins were incubated with the respective protein kinases as described in the legend of Fig. 1. The overall phosphate content was estimated from aliquots which were precipitated and washed on filterpaper (Whatman 3 MM 2 × 2 cm) with 20% (v/v) TCA and after drying counted in a toluene based scintillation cocktail.

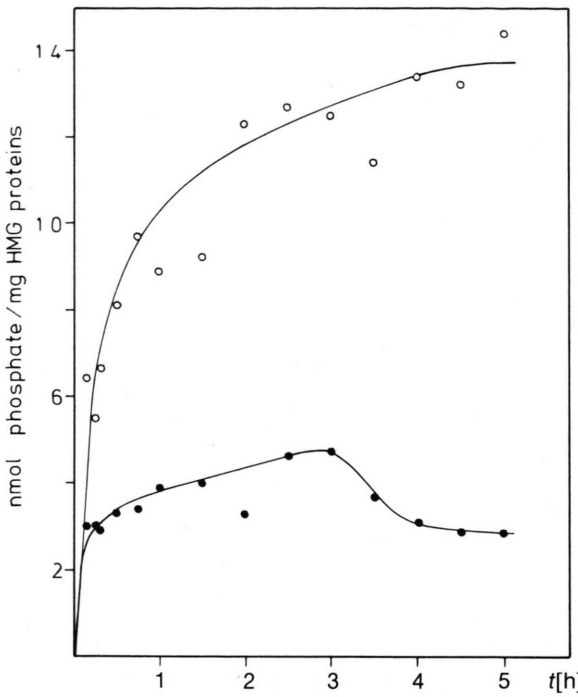

Fig. 1. The time course of phosphorylation of a mixture of HMG proteins by the catalytic subunit of cAMP-dependent protein kinase I from rabbit muscle (○) and a nuclear protein kinase from pig liver (●). In the case of the cytoplasmic enzyme (rabbit muscle) the incubation mixture contained 50 µl buffer (0.1 M Tris-HCl of pH 7.4, 20 mM $MgCl_2$, 2 mM dithiothreitol, 0.6 mM EGTA and 0.6 mM ATP), 40 µl HMG mixture (2–3 mg/ml), 10 µl γ^{32}P ATP (20 Ci/mmol; Amersham Buchler) and 20 µl kinase solution (1 mg/ml). Incubation with the nuclear enzyme was performed in the same manner, however, with a different buffer (40 mM MES of pH 6.9, 16 mM $MgCl_2$ and 0.6 mM ATP) and with a final NaCl concentration of 0.17 M. The incubation was performed at 30 °C. The ^{32}P incorporated was determined as described in Materials and Methods. Each value represents an average of three protocolls, exhibiting good reproducibility.

Results and Discussion

The HMG protein mixture obtained from chicken erythrocyte nuclei was analysed for the relative amount of the four species as described in Material and Methods. HMG-1, 2, 14 and 17 were found in the mixture in the ratio of 4.1:3.6:1.1:1.0; these data related to Coomassie blue staining should reflect a rough mass ratio. Taking into account the molecular weight of these proteins of about 27000 for HMG-1 and HMG-2, about 10000 for HMG-14 and 9250 for HMG-17 [20–22] a mole ratio of 1.41:1.23:1.02:10 can be calculated.

Fig. 1 shows the time course of the phosphate incorporation from ATP into the HMG protein mixture by the two protein kinases. It is obvious that the cytoplasmic enzyme incorporates more than twice as much phosphate as the nuclear enzyme and the phosphorylation by the former enzyme extends over a large time span. With the nuclear enzyme there is a light decrease at the end of the incubation. For the following experiment (Fig. 2) an incubation time of three hours was choosen. Under these conditions on the average 11.2 nmol ^{32}P/mg protein and 6.9 nmol ^{32}P/mg protein was found for the cytoplasmic and the nuclear enzyme, respectively. Using the above mentioned molecular weights and mole ratios, the number of phosphates per HMG protein

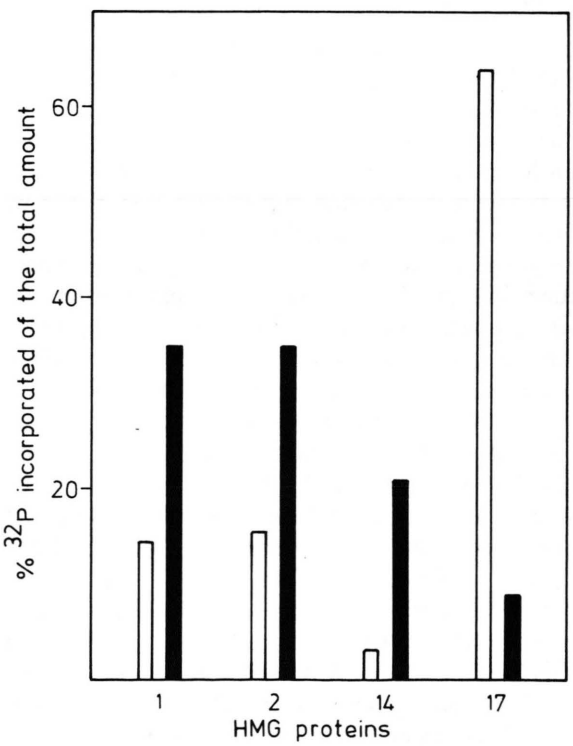

Fig. 2. Relative amounts of ^{32}P incorporated into each of the four HMG proteins by the cytoplasmic kinase (filled bars) and the nuclear protein kinase (open bars). Incubation of the HMG protein mixture with the respective enzyme was performed as indicated in the legend of Fig. 1 for three hours. Appropriate aliquots were applied to a polyacrylamide slab gel electrophoresis containing 0.1% SDS [15]. Staining and destaining was performed as in [27]. The protein bands were cut out from the gel and counted for radioactivity in water. Taking the sum of the counts as 100%, the relative amount of phosphate incorporated was calculated for each HMG component (*cf.* also Ref. [23]).

molecule can be calculated, which are 0.22 for the cytoplasmic and 0.13 for the nuclear enzyme. With a whole histone mixture from erythrocyte nuclei the respective data were found to be 1.2 and 0.06, respectively (H.-A. Arfmann and H. Baydoun, unpublished results). The data indicate that the cytoplasmic enzyme is a rather good histone kinase. The nuclear enzyme is inferior to the cytoplasmic kinase with respect of both substrates, however, it incorporates twice as much phosphate into the HMG proteins than into histones. Obviously this protein kinase is not involved in the histone phosphorylation *in vivo* but is rather a non-histone protein kinase.

Whether the HMG proteins are possible *in vivo* substrates of one of the kinases tested, would become more evident when the specific fraction of ^{32}P incorporated into each component is determined [23]. The cytoplasmic kinase prefers the large HMG proteins HMG-1 and -2, whereas the nuclear kinase clearly indicates a high preference for HMG-17. Considering however, the relative amount of the four proteins within the mixture, the cytoplasmic kinase accepts each component equally well, showing a slight preference for HMG-14. On the other hand, the nuclear enzyme prefers HMG-17, the other HMG proteins are rather bad substrates (Table I). The specificity of kinases is mainly determined by the primary structure of the protein substrate [24], the cytoplasmic kinases preferentially phosphorylate serine or threonine residues in a basic region, whereas the nuclear kinases phosphorylate serine and threonine in a more acidic region [25]. Not only the specificity of the two enzymes, but also the accessibility of the substrates will determine the degree of phosphorylation. As all four HMG proteins contain a high amount of both, basic and acidic amino acid residues, the differences in the phosphorylation of the HMG proteins by the two kinases may be due to different conformations of the four HMG proteins. HMG-1 and HMG-2 have structured regions, whereas HMG-14 and -17 are without structure [26]. The high preference of the nuclear enzyme for HMG-17, however which is associated with chromatin and nucleosomes, may not be accidental, but may have its counterpart *in vivo*. Investigations are underway to determine the *in vivo* phosphorylation of HMG proteins in CHO cells.

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Table I. Relative amount of ^{32}P incorporated into each HMG protein by the cytoplasmic and nuclear kinase. Values are expressed as the percentage phosphate of the total amount for each protein component.

HMG component	Relative amount in the mixture	Cytoplasmic enzyme	Nuclear enzyme
1	4.1	35	15
2	3.6	35	16
14	1.1	21	5.5
17	1	9	64

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