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DIFFERENTIAL PHOSPHORYLATION OF HIGH MOBILITY GROUP PROTEIN HMG 14 FROM CALF THYMUS AND AVIAN ERYTHROCYTES BY A CYCLIC GMP-DEPENDENT PROTEIN KINASE

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Phosphorylation of HMG 14 proteins from calf thymus and avian erythrocytes was studied using a cyclic GMP-dependent protein kinase from bovine lung. HMG 14 from calf thymus was a good substrate for the enzyme, but HMG 14 from avian erythrocytes was not phosphorylated. Of the potential phosphorylation sites, the one in the amino terminal sequence Pro-Lys-Arg-Lys-Val-Ser-Ser-Ala-Glu (residues 1-9) is present in HMG 14 from calf thymus but not in HMG 14 from avian erythrocytes suggesting that the phosphorylated amino acid residue in HMG 14 from calf thymus is Ser-6 (and possibly Ser-7).

The high mobility group (HMG) chromosomal proteins are characterized by high content of acidic and basic residues and solubility in 0.35 M NaCl and 2% trichloroacetic acid (Fig. 1). HMG 14 and HMG 17 bind preferentially to actively transcribed regions of chromatin (1). We have previously shown that a cyclic GMP-dependent protein kinase present in the 0.3 M NaCl extract of avian liver nucleoli efficiently phosphorylates a serine residue in HMG 14 from calf thymus (2). The complete amino acid sequences have been determined for HMG 14 from calf thymus (3) and HMG 17 from calf thymus and avian erythrocytes (4,5). HMG 14 is 100 amino acids long, and HMG 17 is 89 amino acids long. The amino terminal sequence of chicken erythrocyte HMG 14, which is slightly longer (121 amino acids), has also been determined to residue 52 (6,7).

Comparison of the amino acid sequences of these proteins indicates that there are only two potential phosphorylation sites for a serine residue of the type Arg-Arg-X-Ser(P) (8) in the DNA-binding region and one additional site in the amino terminal region of HMG 14 from calf thymus. In HMG 17 from calf thymus, there are only two serine residues that occur in the DNA-binding region of the protein. In this study a cyclic GMP-dependent protein kinase was prepared from bovine lung, and phosphorylation of HMG 14 from calf thymus and avian erythrocytes was compared in vitro.

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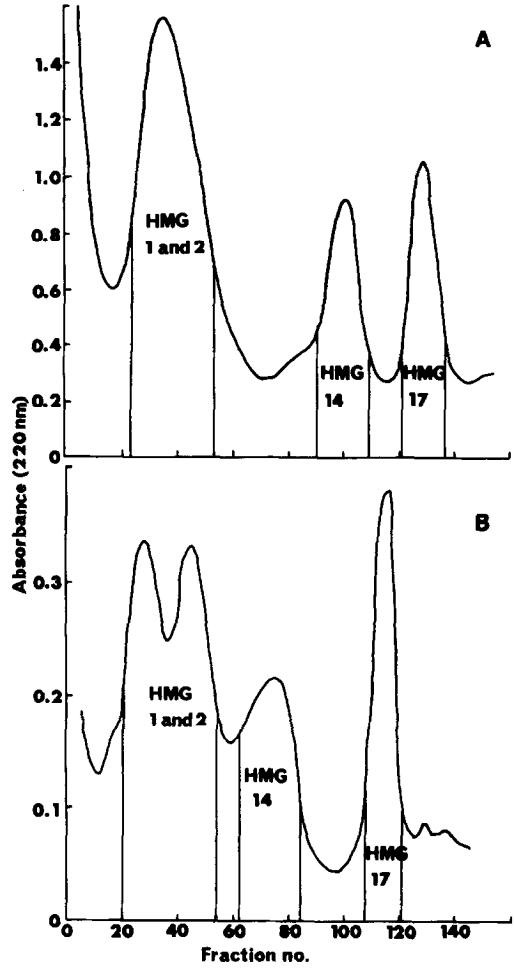

Figure 1. Chromatography of HMG proteins from calf thymus (A) and rooster erythrocytes (B) on CM-Sephadex C-25. The column was eluted at 4°C with 2.0 l of a linear gradient of 0.1 to 0.6 M NaCl containing 7.5 mM sodium borate (pH 8.8). Absorbance at 220 nm was measured from the 10-ml fractions. The HMG proteins were pooled and lyophilized as indicated by vertical lines.

EXPERIMENTAL PROCEDURE

White Leghorn roosters were obtained from a local hatchery and fed ad libitum with a standard diet. Calf thymus and bovine lung were obtained from a local slaughterhouse. [^{32}P]ATP (3000 Ci/mmol) was purchased from the Radiochemical Centre, Amersham. Cyclic GMP was obtained from Sigma Chemical Co. Sodium dodecyl sulfate was obtained from Fluka A.G. and NCS solubilizer from Amersham/Searle Co. Reagents for polyacrylamide gel electrophoresis were from Eastman Kodak Co. CM-Sephadex C-25 was the product of Pharmacia Fine Chemicals. Whatman DE-52 cellulose was purchased from Whatman Ltd., and 8-(6-aminohexylamino)cAMP-agarose was from P-L Biochemicals Inc.

HMG 14 and HMG 17 from calf thymus and rooster erythrocytes were purified as described previously (6,9). Typical elution profiles on CM-Sephadex C-25 are shown in Fig. 1. The purity of the isolated HMG proteins was checked by polyacrylamide gel electrophoresis in 15% gels containing 0.1% SDS according to Weber and Osborn (10). The cyclic GMP-dependent protein kinase was purified from bovine lung using a

standard procedure essentially as described by Walter et al. (11). Briefly, the soluble extract from bovine lung was fractionated on DEAE-cellulose, concentrated, passed through a 8-(6-aminohexylamino)cAMP-agarose column and eluted with 10 mM cAMP.

The standard mixture for phosphorylation assay contained in a total volume of 0.1 ml: 0.05 mg of substrate protein, 50 mM glycerol phosphate (pH 7.5), 0.3 mM EGTA, 2 mM theophylline, 10 mM $MgCl_2$, 17 nM [^{32}P] ATP, 1 µM cGMP and 50 µl of the purified protein kinase. Control incubations were performed without cGMP. The reaction was initiated by the addition of [^{32}P] ATP and carried out for 10 min at 37^{0}C. The reaction was terminated, and an aliquot was subjected to electrophoresis as described in the legend to Fig. 2. Protein was determined as described by Spector (12).

RESULTS AND DISCUSSION

Previously, a chromatin protein with a molecular weight of about 11 000 was found to be phosphorylated in vitro by a cGMP-dependent protein kinase loosely bound to nucleoli in avian liver (13). In subsequent studies we identified the substrate as HMG 14 and found that the phosphorylated amino acid residue was serine (2). Bhorjee (14) has recently reported that HMG 14 is specifically phosphorylated in synchronized HeLa cells during the G_2 phase. Levy-Wilson (15) has found that butyrate treatment, which causes hyperacetylation of histones and enhances the DNase I sensitivity of chromatin, also promotes hyperphosphorylation of the HMG 14 and HMG 17 in HeLa cells. In addition, Paulson and Taylor (16) have demonstrated phosphorylation of HMG 14 by an endogenous kinase present in metaphase chromosomes from isolated HeLa cells and Saffer and Glazer (17) have found that a growth-associated phosphorylation of HMG 14 occurs in Ehrlich ascites cells and that HMG 14 is also phosphorylated in L1210 and P388 leukemia cells, human colon carcinoma cells and Chinese hamster ovary cells.

In the studies mentioned above, regulation of phosphorylation by cyclic nucleotides was not studied. Cooper et al. (18) have recently shown that TSH stimulates ^{32}P-labeling of HMG 14 in thyroid slices, which may indicate regulation through cAMP. In a detailed study, Walton et al. (19) reported the phosphorylation of HMG 14 and HMG 17 from calf thymus by a cGMP-dependent protein kinase prepared from bovine lung and by the catalytic subunit of a cAMP-dependent protein kinase prepared from bovine heart. They found that only HMG 14 contains a major high affinity site, which can be phosphorylated by both types of kinases (preferentially by the cGMP-dependent kinase) and that the phosphorylated amino acid residue in HMG 14 is Ser-6. In addition, HMG 14 and HMG 17 were shown to contain a minor and homologous site of phosphorylation by the cGMP-dependent kinase (Ser-24 and Ser-28, respectively).

The present results (Fig. 2) indicate that the well-studied cGMP-dependent protein kinase from bovine lung is similar to the enzyme associated with nucleolar chromatin in avian liver, since it phosphorylates HMG 14 from calf thymus efficiently (2). In contrast, HMG 14 from avian erythrocytes showed essentially no phosphorylation (Fig. 2). Comparison of the amino acid sequences of HMG 14 and HMG 17 from

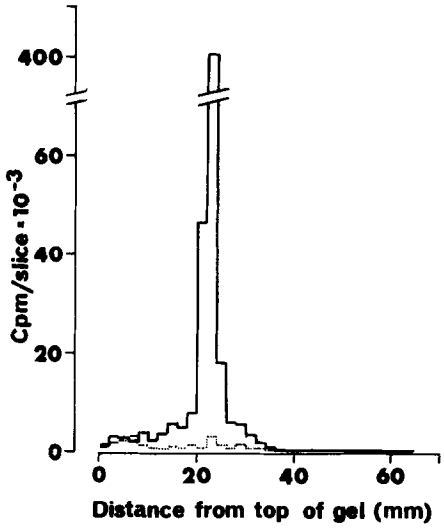

Figure 2. Analysis of phosphorylated HMG 14 proteins from calf thymus (solid line) and rooster erythrocytes (dotted line) by sodium dodecyl sulfate polyacrylamide gel electrophoresis. 0.05 mg of calf thymus HMG 14 and 0.05 ml of rooster erythrocyte HMG 14 were phosphorylated separately in a standard incubation mixture in the presence of 1 µM cGMP. The reaction was terminated by the addition of 25 µl of 5% sodium dodecyl sulfate, and the tubes were heated at 100°C for 5 min. Aliquots of the samples were subjected to electrophoresis in 15% cylindrical polyacrylamide gels containing 0.1% sodium dodecyl sulfate. The electrophoresis was performed at 6 mA/tube using Bromphenol blue as a tracking dye. After electrophoresis, the gels were cut and 2-mm slices were placed in separate counting vials and incubated overnight at 37°C with 0.1 ml of water and 0.9 ml of NCS-solubilizer. The slices were then counted for radioactivity using 10 ml of a toluene-based counting fluid. Parallel gels with HMG 14, which had not been incubated, were stained after electrophoresis with 0.25% Coomassie brilliant blue in 50% methanol and 7% acetic acid. The gels were destained with methanol and 7% acetic acid.

calf thymus with the homologous proteins from avian erythrocytes suggests that the major difference with respect to potential phosphorylation sites by cyclic nucleotide-dependent protein kinases of the type Arg-Arg-X-Ser(P) (8) occurs in the amino terminal region of HMG 14. This sequence is Pro-Lys-Arg-Lys-Val-Ser-Ser-Ala-Glu (residues 1-9) in HMG 14 from calf thymus, whereas in HMG 14 from chicken erythrocytes the corresponding sequence is Pro-Lys-Arg-Lys-Ala-Pro-Ala-Glu (residues 1-8) (3,6). Thus, the present results agree with the recent results of Walton et al. (19) and suggest that the major high affinity site in HMG 14 from calf thymus, which is phosphorylated by the cGMP-dependent protein kinase from bovine lung or nucleolar chromatin, is Ser-6 (and possibly Ser-7).

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