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PHOSPHORYLATION ALTERS THE AFFINITY OF HIGH MOBILITY GROUP PROTEIN HMG 14 FOR SINGLE-STRANDED DNA

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The effect of phosphorylation on the affinity of HMG 14 from calf thymus for single-stranded DNA (ssDNA) was studied, using a cyclic GMP-dependent protein kinase from bovine lung and a nuclear protein kinase II from rat liver. When phosphorylated by G-kinase, HMG 14 eluted at 0.27 M NaCl from the ssDNA-column, whereas the native protein eluted at 0.30 M salt concentration. In contrast, phosphorylation by nuclear protein kinase II did not alter dissociation of HMG 14 from ssDNA and the phosphoprotein consequently coeluted with the native HMG 14. Thus, addition of a negative charge by phosphorylation of the Ser-6 residue by G-kinase presumably weakens the interaction between the DNA-binding amino acids of HMG 14 and the negatively charged phosphate groups of DNA.

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. They are universally present in the eukaryotic kingdom and during the last decade the primary structures of the four major HMG proteins (HMG 1, 2, 14 and 17) from calf thymus have been determined (1). Furthermore, several post-synthetic modifications of the HMG proteins including acetylation, methylation, phosphorylation and ADP-ribosylation have been described (2).

The functions of the HMG proteins are not known. There are about 10^6 HMG protein molecules per mammalian cell nucleus (3), and obviously they are structural elements. HMG 14 and HMG 17 are clearly proteins associated with nucleosome core particles (3). Since they seem to be preferentially associated with transcriptionally active chromatin, they are thought to organize the structure of chromatin so that gene transcription can occur (4). HMG 14, particularly, has been suggested to be involved in maintaining the structure of active chromatin (4). NMR studies on the binding of calf thymus HMG 14 to DNA have shown that the DNA binding region of HMG 14 is located between residues 17-60 (1). Since HMG 14 lacks a globular structure, phosphorylation

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most likely affects its interaction with DNA and/or chromatin proteins than alters the folding of HMG 14 (2).

HMG proteins share the property of being selectively retained by a single-stranded DNA column at 0.2 M NaCl/1 mM Tris-Cl (pH 7.5) (6). We have taken advantage of this property to examine the influence of phosphorylation on the affinity of HMG 14 for DNA.

EXPERIMENTAL PROCEDURE

Adult male and female rats (Wistar BD-IX) were obtained from the Experimental Animal Center of the University of Kuopio and fed ad libitum with a standard diet. Calf thymus and bovine lung were obtained fresh from a local slaughterhouse. [γ -^{32}P] ATP (3 Ci/mmol) was purchased from the Radiochemical Centre, Amersham, U.K. Single-stranded DNA-agarose was purchased from Bethesda Research Laboratories and 8-(6-aminohexylamino)cAMP-agarose was the product of P-L Biochemicals Inc. CM-Sephadex C-25 and Sephadex G-25 were purchased from Pharmacia Fine Chemicals, Uppsala.

HMG 14 was purified from calf thymus by chromatography on CM-Sephadex C-25 as described earlier (7). Nuclear protein kinase II from rat liver and cGMP-dependent protein kinase from bovine lung were purified as described previously (8, 9).

0.3 mg of HMG 14 was phosphorylated by cGMP-dependent protein kinase (4 µg) or nuclear protein kinase II (8 µg) in the presence of 10 µM [^{32}P] ATP for two hours at 30°C in the standard assay mixtures described earlier (10). Free radioactivity was then separated from the phosphoprotein by chromatography on Sephadex G-25 (1 x 25 cm). Protein was eluted with 0.2 M NaCl in 1 mM Tris-Cl, pH 7.5. 0.7 mg of native HMG 14 was mixed with the phosphorylated protein and the sample was applied to a ssDNA-agarose column (total volume 10 ml) containing 7 mg of calf thymus ssDNA pre-equilibrated with the starting buffer (0.2 M NaCl in 1 mM Tris-Cl, pH 7.5). The column was washed with one bed volume of the starting buffer and the proteins were eluted with a linear gradient of 0.2-0.6 M NaCl containing 1 mM Tris-Cl, pH 7.5. Protein was detected by measuring absorbance at 220 nm or radioactivity as Cerenkov radiation. Protein concentration was determined as described by Spector (11).

RESULTS AND DISCUSSION

The elution profiles from the ssDNA-agarose column of native HMG 14, HMG 14 phosphorylated by G-kinase (A) or by nuclear protein kinase II (B) are shown in Fig. 1. The substrate protein was not dephosphorylated before use, since it has been reported that prolonged incubation of the HMG proteins with alkaline phosphatase does not enhance their total phosphorylation by G-kinase (12). The native HMG 14 eluted from the column at 0.30 M NaCl in both experiments. Interestingly, HMG 14 phosphorylated by G-kinase eluted at a considerably lower ionic strength of 0.27 M NaCl. However, when phosphorylated by nuclear protein kinase II, HMG 14 coeluted with the native protein. Because of possible degradation of HMG 14 during incubation and chromatography, the phosphorylated protein was examined by acetic acid/urea polyacrylamide gel electrophoresis (10) and found to migrate identically with authentic HMG 14 (data not shown).

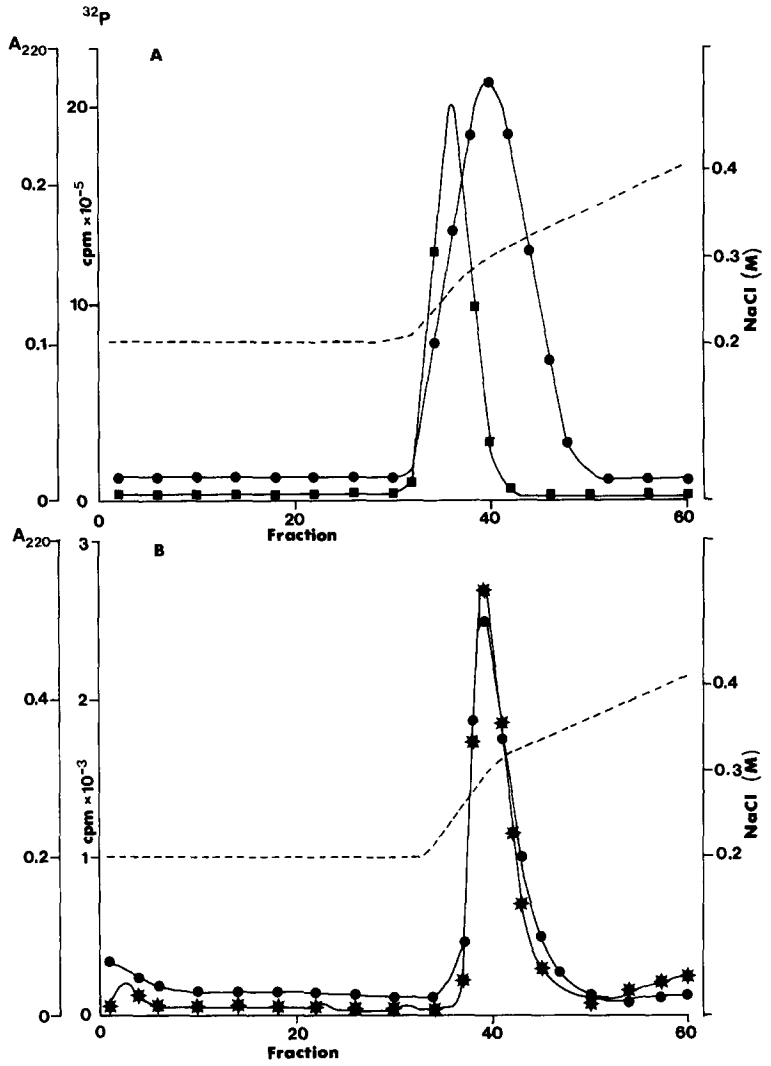

Figure 1. Chromatography of native HMG 14 (●) and HMG 14 phosphorylated by G-kinase (■) or by nuclear protein kinase II (✱) on ssDNA-agarose. The column was eluted at 4°C with 100 ml of a linear gradient of 0.2-0.6 M NaCl containing 1 mM Tris-Cl, pH 7.5. Absorbance at 220 nm was measured from 1 ml fractions and radioactivity was measured as Cerenkov radiation. The salt concentration in the gradient fractions was determined by measuring the conductivity at room temperature with a Philips PW 9501/01 conductivity meter.

We have previously reported phosphorylation of HMG 14 from calf thymus by G-kinase from bovine lung (13). We have also found that the phosphorylated amino acid residue in HMG 14 is Ser-6 (9). A minor site of phosphorylation by G-kinase (Ser-24) has also been shown to exist (12). Nuclear protein kinase II, on the other hand, catalyzes phosphorylation of Ser-89 at the carboxy-terminal region of the molecule (14). Phosphorylation of HMG 14 increases its negative charge. The present results indicate that

phosphorylation of Ser-6 by G-kinase weakens the interaction of the DNA-binding segment of HMG 14 with the negatively charged phosphate groups of DNA. Phosphorylation of Ser-89, in contrast, does not alter the binding of HMG 14 to DNA.

Various regions of the HMG 14 molecule seem to have different functions (15). The amino-terminal region is very basic and it interacts with DNA. It seems reasonable to suggest that covalent modifications affecting the net charge near or at this region (caused e.g. by phosphorylation) influence the binding of HMG 14 to DNA. The acidic carboxy-terminal region of the molecule presumably interacts with other chromosomal proteins (1) and the function of phosphorylation of Ser-89 in HMG 14 might be connected with these processes.

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