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BINDING OF NON-HISTONE CHROMOSOMAL PROTEIN HMG1 TO HISTONE H3 IN NUCLEOSOMES DETECTED BY PHOTOCHEMICAL CROSS-LINKING

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The interaction of non-histone chromosomal protein HMG1 with core histones in nucleosomes was studied via reconstitution and photochemical cross-linking. The results obtained indicated that photoaffinity-labeled HMG1 interacted in nucleosomes with histone H3. Similar experiments with peptides derived from HMG1 by V8 protease digestion allowed to identify N-terminal domain of HMG1 (peptide V3) as a binding region for histone H3 in nucleosomes.

High-mobility-group (HMG) proteins are relatively abundant non-histone chromatin-associated proteins (1). Two distinct groups of HMG proteins were identified in calf thymus, HMG14 and HMG17 (M_r 9 000 - 10 500), and HMG1 and HMG2 (M_r 26 000 - 29 000). The function of HMG1 and HMG2 in vivo is not known but several reports indicated their possible importance as structural components of chromatin required during transcription or replication (2-4).

HMG1 and HMG2 are highly homologous in sequence with a large number of both basic (25%) and acidic (30%) amino acid residues (5) asymmetrically distributed into three structural domains (6,7). The existence of different functional domains within HMG1 and HMG2 may explain the ability of these proteins to interact both with DNA and with histones (8-14).

Recently we have reported the detection of HMG1 (HMG2) - core histone interactions in nucleosomes reconstituted with HMG1 and HMG2 (13). The aim of the present paper was to ascertain which of the core histones in nucleosomes interacted with HMG1, and to identify the binding domain of HMG1 for the respective core histone in nucleosomes.

ABBREVIATIONS:
HMG: high mobility group; SADP: N-succinimidyl(4-azidophenyl)-1,3-dithiopropionate; DFP: diisopropylfluorophosphate; V8 protease; Staphylococcus aureus serine proteinase, EC 3.4.21.19.

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MATERIALS AND METHODS

Preparation of nucleosomes: H1,H5-depleted nucleosomes were isolated from chick erythrocyte nuclei as described by Shick et al. (15), with the modifications mentioned previously (13,14).

Isolation of HMG1: HMG1 protein was isolated from calf thymus under non-denaturing conditions as described by Marekov et al. (16) with some modifications (13).

Radioiodination of HMG1: HMG1 (1-2 mg/ml) was ^{125}I-labeled in 0.2 M NaCl/0.1 M sodium borate (pH 7.5) using Na^{125}I and IODO-BEADS (kindly provided by Pierce) essentially as described by Markwell (17).

Photoaffinity labeling of HMG1: all procedures were carried out in dark room with a safety lamp plus a green filter (No. 570, FOMA, Czechoslovakia). ^{125}I-HMG1 (1.5 mg/ml) was mixed in 0.2 M NaCl/0.1 M sodium borate (pH 7.5)/0.2 mM DFP with 0.025 vol. SADP (Sigma), freshly dissolved in acetonitrile at a concentration of 35 mg/ml. The reaction mixture was kept at room temperature for 2 hours. Unreacted SADP was quenched with 0.1 vol. 1 M glycine (pH 7.5)/0.5 mM DFP at 37 °C for 60 min.

Digestion of HMG1 with V8 protease: SADP derivative of ^{125}I-HMG1 (1 mg/ml) was digested in 0.2 M NaCl/0.1 M sodium borate (pH 7.5) with V8 protease (580 U/mg, kindly provided by ICN Biomedicals) for 12 min at 37 °C. The ratio of HMG1/V8 protease (w/w) was 100:1. Digestion was terminated by the addition of 0.1 vol. 0.5 M DFP in 0.1 M sodium borate (pH 7.5) and further incubation for 60 min at 37 °C (caution, DFP is a severe poison!), followed by the addition of a few microliters of 2 M NaOH to readjust the pH.

Reconstitution: the nucleosomes (0.2 - 0.3 mg DNA/ml) were admixed with undigested or digested SADP derivative of ^{125}I-HMG1 (two HMG1 molecules per nucleosome) in 0.35 M NaCl/50 mM sodium borate (pH 7.5)/0.2 mM EDTA and dialyzed in a test tube (covered with Spectra/Pore dialyzing membrane, cut-off 2 000) against 0.1 M NaCl/50 mM Tris.HCl (pH 7.8)/0.2 mM EDTA/0.2 mM DFP.

Photochemical cross-linking: the cross-linking reaction was initiated by irradiation of the samples for 5 sec under an ozone-free high-pressure 1000W Xenon UV-lamp (Oriel, U.S.A.) emitting UV-light of wavelengths longer than 260 nm.

Gel electrophoresis: the irradiated samples were twice extracted with 0.25 M HCl, then precipitated with 25% (w/v) trichloroacetic-acid, and finally washed several times in acetone. The sediments were air-dried and dissolved in electrophoretic sample buffer according to Laemmli (18), in which 2-mercaptoethanol was omitted. Cross-linked proteins were separated in the first dimension in the absence of any reducing agents on an SDS/15% polyacrylamide slab gel (1 mm thick) using the buffer system of Laemmli (18). Cleavage of the cross-links by 2-mercaptoethanol and two-dimensional electrophoresis on an SDS/15% polyacrylamide slab gel (1.5 mm thick) were performed as previously described (13). Gels were stained by silver (19), dried and subjected to autoradiography at -70 °C with an intensifying screen.

RESULTS

Interaction of HMG1 with histone H3 in nucleosomes

The interaction of HMG1 with core histones in nucleosomes was recently detected in reconstitution experiments using chemical cross-linking (13). This technique, however, lead to a very complicated cross-linking pattern, and thus it was difficult to ascertain which of the core histones was cross-linked with HMG1 or HMG2 (13). In order to identify the contribution of each of the core histones in nucleosomes to the interaction with HMG1, a photochemical cross-linking assay (for review see 20) was employed in the present paper. This approach prevented an exhaustive cross-linking and resulted in a simpler cross-linking pattern. As shown in Fig. 1, UV-irradiation of the free SADP derivative of ^{125}I-HMG1 produced several low-mobility bands corresponding to multiple contacts between individual HMG1 molecules - dimers, trimers, etc. (**Fig. 1, lane 2**). On the other hand, UV-irradiation of SADP derivative of ^{125}I-HMG1 reconstituted with nucleosomes resulted in the appearance of additional low-mobility bands, some of them moving close to HMG1 oligomers (**Fig. 1,, lane 3**). The most intensive cross-linked product in **Fig. 1, lane 3** (denoted by a void arrow-head), as well as the other cross-linked products (marked by solid arrow-heads) were resolved on a two-dimensional gel into HMG1 and histone H3 as revealed by sensitive silver staining (**Fig.2 A**). No other core

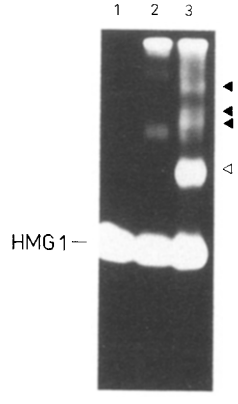

Figure 1. Autoradiogram of one-dimensional gel electrophoresis.
(1), free ^{125}I-HMG1,
(2), UV-irradiated SADP derivative of ^{125}I-HMG1,
(3), UV-irradiated nucleosomes reconstituted with SADP derivative of ^{125}I-HMG1.
Void and solid arrow-heads mark the positioning of major and minor HMG1 - histone H3 complexes, respectively.

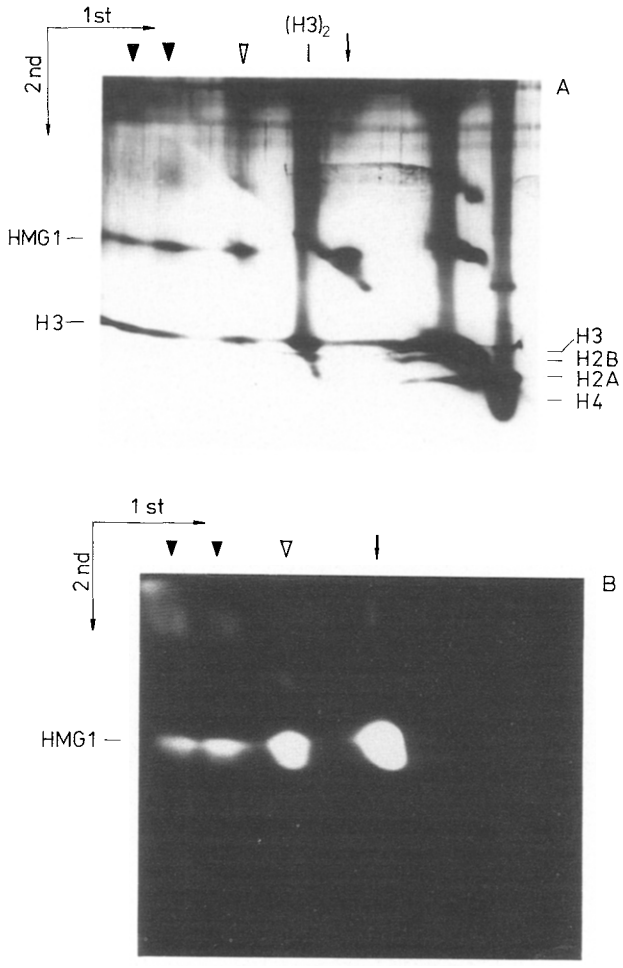

Figure 2. Two-dimensional gel electrophoresis of proteins after UV-irradiation of nucleosomes reconstituted with SADP derivative of ^{125}I-HMG1.
A: silver staining.
B: autoradiography.
Void and solid arrow-heads indicate the positioning of major and minor HMG1 - histone H3 complexes, respectively. The arrow marks the mobility of free HMG1.

histones were found to be cross-linked with HMG1 under the experimental conditions described above (see also Discussion). The presence of HMG1 among the cleaved cross-linked products was also verified by autoradiography of the two-dimensional gel (**Fig. 2 B**).

The appearance of an $(H3)_2$ dimer in **Fig. 2** could be related to the oxidized state (the reaction conditions prior to electrophoresis in the second direction were not reductive) of the cysteine residues (intermolecular cross-links between two H3

histones since chick erythrocyte histone H3 has a single cysteine (21)).

Identification of binding domains of HMG1 for histone H3 in nucleosomes

HMG1 protein has a three-domain structure (6,7). To identify the contribution of each of the domains of HMG1 to the interaction with histone H3 in nucleosomes, experiments were performed with peptides produced by limited cleavage of HMG1 with V8 protease from Staphylococcus aureus. As described by Carballo et al. (22), V8 protease splits HMG1 into three main peptides - V3(N-terminal domain, residues 1-74; M_r 11 500), V2 (central domain, residues 80-185; M_r 13 000), and V1 (central domain attached to C-terminal domain, residues 74-243; M_r 18 000). In this paper the digestion of HMG1 with V8 protease was very mild so that in addition to the main peptides V1, V2 and V3, a little amount of undigested HMG1 and peptide X was detected as well. Peptide X represented most probably the autolytic product of HMG1, denoted by Goodwin and colleagues (23) as HMG3 (residues 1-183, i.e. N-terminal plus central domain).

The experimental strategy for the identification of the binding domains of HMG1 for histone H3 in nucleosomes was similar to that used by Bernués et al. (10) for the study of the interaction of HMG1 at the domain level with isolated core histone oligomers. Briefly, ^{125}I-HMG1, modified with SADP, was partially digested with V8 protease and subsequently reconstituted with nucleosomes. The results obtained are displayed in **Fig. 3**. Open arrow-heads on a silver stained two-dimensional gel (**Fig. 3 A**) mark an intensive spot corresponding to peptide V3, and faint spots corresponding to peptides V2 and V1, all of them previously photochemically cross-linked with histone H3. Identification of the HMG1 peptides among the cross-linked products with histone H3 was also performed by autoradiography of the gel shown in **Fig. 3 A**. Due to the overexposition, the spots corresponding to peptides V2 and V1 (previously cross-linked with histone H3) appeared on the autoradiogram (**Fig. 3 B**) more intensive than one would expect from the silver staining.

DISCUSSION

The experiments described in this paper revealed that the photoaffinity-labeled HMG1 could interact in nucleosomes with

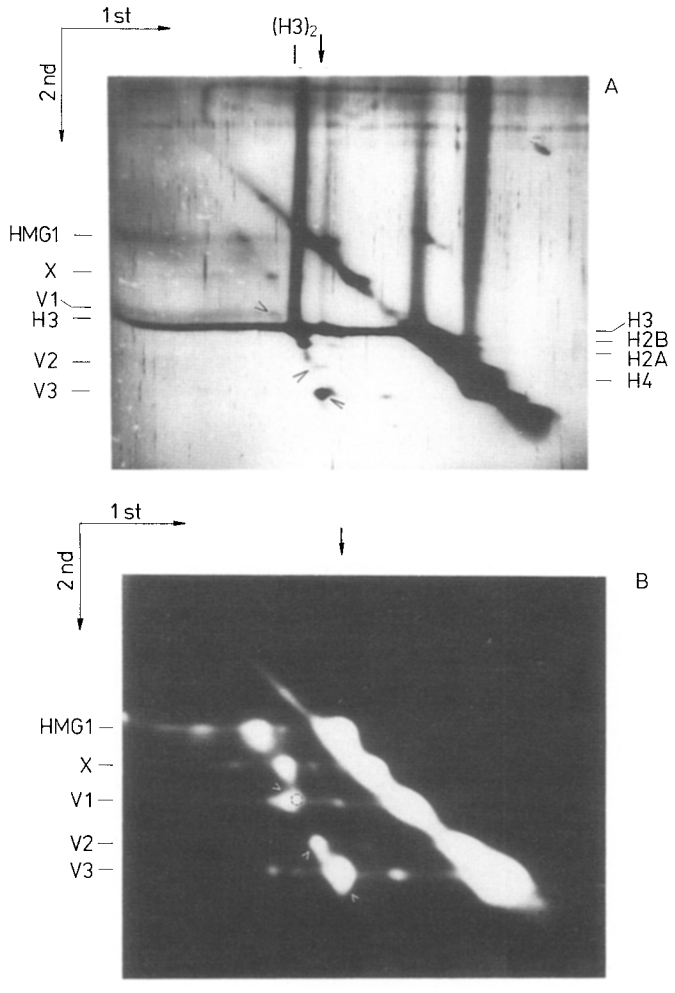

Figure 3. Two-dimensional gel electrophoresis of proteins after Uv-irradiation of nucleosomes reconstituted with SADP derivative of ^{125}I-HMG1 peptides.
A: silver staining.
B: autoradiography.
Open arrow-heads indicate the positioning of HMG1 peptides previously photochemically cross-linked in nucleosomes with histone H3. The arrow marks the mobility of free HMG1.

histone H3. The apparent inability of the photoaffinity-labeled HMG1 to interact in nucleosomes with other core histones might be explained as follows. The presence of a photoaffinity label on lysine and arginine residues of HMG1 could prevent the establishment of interactions between HMG1 and histones H4, H2A and H2B. Alternatively, the photoaffinity label may not be close enough to the hypothetical binding sites of HMG1 for histones H4, H2A or H2B. Another explanation might be the different accessibility of the individual core histones in the nucleosome.

The above-mentioned factors could then elucidate the fact (Štros, preliminary results) that when core histones in nucleosomes were photoaffinity labeled instead of HMG1, HMG1 was found to be cross-linked not only with histone H3 but also with histones H2A and H4. The latter finding with HMG1-containing nucleosomes supports the previously published results of Bernués et al. (10) describing the detection of interactions between HMG1 and an isolated H2A-H2B dimer or an $(H3\text{-}H4)_2$ tetramer in free solution.

The experiments in this paper further indicated that the domain of HMG1 interacting in nucleosomes with histone H3 was the N-terminal (peptide V3). Similar results were recently presented with HMG1 and isolated $(H3\text{-}H4)_2$ tetramers (10). Whether apart from the N-terminal domain also other domains of HMG1 could be involved in nucleosomes in the interaction with histone H3 (as might be suggested from **Fig. 3**), remains unclear. Using short-pulse photolysis - to minimalize the probability of formation of complexes by random collisions - could clarify this question.

The biological relevance of HMG1 - core histone interactions is not yet known. However, Bonne-Andrea et al. (24) recently reported that HMG1 could mediate chromatin assembly in vitro. Although the exact mechanism of this process is not clear, it is obvious that such functioning of HMG1 would require a number of interactions between HMG1 and the other chromatin components such as DNA and histones.

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