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In Vivo Acetylation of HMG1 Protein Enhances Its Binding Affinity to Distorted DNA Structures

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ABSTRACT: The postsynthetic acetylation of HMG1 protein has been known for more than 20 years, but the effect of this modification on the properties of the protein has not been studied so far. Acetylated HMG1 was isolated from cells grown in the presence of sodium *n*-butyrate and identified as a monoacetylated protein, modified at lysine 2. Acetylated and parental forms of HMG1 were compared relative to their binding affinity to distorted DNA structures. By using mobility shift assay to determine the dissociation constants, we show that acetylation enhanced the ability of HMG1 to recognize UV light- or cisplatin-damaged DNA and four-way junctions. Since the modified lysine lies adjacent to the HMG1 DNA-binding domain, the results obtained were attributed to acetylation-induced conformational change in HMG1. The potential role of acetylation in modulating the interactions of HMG1 with both damaged DNA and other proteins is discussed.

High mobility group (HMG)[1] proteins are a heterogeneous set of nonhistone chromosomal proteins whose function is obscure despite their abundance and ubiquity and more than 25 years of intensive studies (*1*). A subset of these proteins contains the so-called HMG box domain, a DNA-binding motif that confers them the peculiar feature to recognize bent DNA or to induce bending in linear duplex DNA (*2*−*4*). The most abundant of the HMG box proteins are HMG1 and 2. They bind preferentially to distorted DNA structures such as four way junctions (*5*), cisplatin- and UV-damaged DNA (*6*−*8*), and semicatenated DNA loops (*9*). HMG1 was reported to kink the double helix (*10*−*12*) and to constrain negative supercoils in plasmid DNA (*13*, *14*).

HMG1 from eukaryotes is composed of one or two flat L-shaped HMG domains (*15*−*17*), which are amino- and/or carboxyl-terminally flanked by stretches of positively or negatively charged residues. Although DNA binding occurs primarily through the HMG domain, the flanking regions and particularly the COOH-tail have been shown to alter the affinity of the protein to DNA (*8*, *14*, *18*−*22*). Moreover, the identification in the nucleus of a specific protease that cleaves the C-terminal portion of HMG1 (*23*) suggests a means for in vivo modulation of HMG1−DNA interactions. Another possibility to influence the DNA-binding properties of HMG1 resides in the postsynthetic modifications of the protein. It has already been shown that phosphorylation of HMG1 from dipteran insects Chironomus and Drosophila is important for the proper folding of the protein and its DNA-binding specificity (*24*, *25*). Another modification of HMG1 is the reversible acetylation of lysines at positions 2 and 11 in the N-terminal region of the protein (*26*, *27*). Although this modification has been known for more than two decades, the consequences for the properties of the protein have not been studied. A possibility to address the last question is provided by the earlier report that sodium butyrate inhibits the enzymatic deacetylation of both histones and HMG1 (*27*). We used this finding to generate in vivo acetylated HMG1 protein. Having purified and identified the modified protein, its DNA-binding specificity was analyzed with UV damaged- and cisplatinated DNA and with synthetic four-way junctions as well. Binding affinity of acetylated HMG1 protein to these DNA structures was significantly higher than that of the unmodified parental protein.

EXPERIMENTAL PROCEDURES

Preparation of Acetylated HMG1 Protein. Guerin ascites tumor cells were inoculated in albino rats. On day 7 after transplantation, the ascites fluid was collected and directly suspended in a HEPES modification of DMEM (Sigma) containing 5% calf serum, streptomycin (100 μg/mL), heparin (0.5 unit/mL), and 20 mM sodium *n*-butyrate. Butyrate was added to all buffers used in subsequent isolation and handling of HMG1 at a final concentration of 10 mM. The cells were incubated 12 h at 37 °C under gentle shaking. The viability of the cells was controlled by the trypan blue exclusion test. After incubation, the cells were pelleted and further processed to isolate nuclei as described previously (*28*). Isolation and purification of HMG1 protein was performed by the non-denaturing salt extraction method (*29*). Following chromatography on a DEAE-Sephacel column, eluted fractions were analyzed on polyacrylamide gel containing urea and Triton (*30*). Fractions containing HMG1 were pooled, dialyzed against 50 mM Tris-HCl, pH 7.4, 20 mM NaCl, 10 mM sodium butyrate, and concentrated on Amicon membranes.

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[1] Abbreviations: EMSA, electrophoretic mobility shift assay; 4WJ, four-way junction DNA; HMG, high mobility group.

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The final sample was loaded on a second DEAE-Sephacel column. Elution was carried out using sloping gradient and collecting 200 μL fractions. Exactly the same procedure was applied to isolate nonmodified HMG1 except that butyrate was omitted from the medium. Electrophoresis in SDS-containing polyacrylamide gels was performed as described (*31*). Immunoblotting was carried out following previously reported procedure (*8*) except that proteins were transferred from Triton-urea gels to hydrophobic membranes (Amersham). Polyclonal antibody to HMG1 was prepared and immunospecifically purified by affinity chromatography as described elsewhere (*32*). Concentrations of HMG1 were routinely determined using the Coomassie reagent with calf thymus HMG1 as a standard (*33*).

Preparation of UV-Damaged Oligonucleotide. All synthetic single-stranded oligonucleotides used in this study were ordered from GENESET. They were purified on a 15% sequencing gel prior to preparing of the probes. DNA concentration of all stock solutions used was calculated from the absorbance at 260 nm. The generation of UV-damaged oligonucleotide was described previously (*8*). It contains a single site for formation of pyrimidine dimer upon irradiation with UV light (the adjacent pyrimidines are underlined): 5′-ACG CAT GCA TGC A**TT** GCG CGC GTA TA-3′. ^{32}P-End-labeling of both strands and annealing and purification of the final double-stranded oligonucleotide as well as the conditions for irradiation with UV light have been detailed previously (*8*).

Preparation of Oligonucleotide Containing Cisplatin Adducts. To precisely and easily quantify the lesions generated upon treatment with cisplatin, we employed a site-specifically platinated oligonucleotide. This was achieved by using chemically synthesized 40 bp oligonucleotide in which a single GpG for adduct formation was inserted in a restriction site for *Bam*H1 endonuclease (marked by bolt letters): 5′ CGC TAT GCG TAC GTA TT**G GAT CC**T TAT GCG TTA CTG TAT G-3′. The strand was allowed to react with cisplatin at a drug/nucleotide molar ratio 20:1 in TE buffer (10 mM Tris, 1 mM EDTA), pH 7.4, in the dark at 37 °C for 24 h. The unbound drug was removed by ethanol precipitation. The two complementary strands were end-labeled with [γ-^{32}P]dATP and T4 polynucleotide kinase (Amersham Pharmacia Biotech) and annealed, and the resulting double-stranded oligonucleotide purified as described (*8*). Formation of cisplatin adduct makes the restriction site inaccessible for the enzyme, so that the level of platination of GpG sites upon cisplatin treatment was easily controlled by digestion of the [^{32}P]-labeled probe with *Bam*HI and subsequent electrophoresis in native gel. At the above-described conditions for platination, the original 40 bp oligonucleotide was observed only, indicating a modification of all existing GpG.

Preparation of a Four-Way Junction DNA (4WJ). It was constructed as described (*34*) using the following four sequences (5′-3′): GTCGAATTCAGCACGAGTCCTAACGC-CAGATCTGGC; GGGCCAGATCTGGCGTTAGGTGAT-ACCGATGCATCGGC; GCCGATGCATCGGTATCGCT-TACGACTAGTGAG; GGCTCACTAGTCGTAAGCCAC-TCGTGCTGAATTCGAC.

The 4WJ was assembled as follows: 2 pmol of a ^{32}P-labeled oligonucleotide was mixed in 10 mM Tris-HCl, pH 8, 100 mM NaCl, and 1 mM EDTA with the three cold oligonucleotides (3 pmol each) in a final volume of 50 μL. The reaction was heated for 5 min at 98 °C, cooled slowly to 37 °C, allowed to anneal at this temperature for at least 6 h and then cooled to room temperature. The efficiency of 4WJ DNA assembly was checked on 8% native polyacrylamide gel.

Electrophoretic Mobility Shift Assay (EMSA). DNA-binding assays of both acetylated and nonmodified HMG1 protein with the ^{32}P-labeled probes (UV- or cisplatin-damaged oligonucleotides and four way junction) were performed as described previously (*8*) except that the incubation mixture was supplemented with 10 mM sodium *n*-butyrate. Nonlabeled sonicated salmon sperm DNA was added as competitor in all experiments except those designed to determine dissociation constants. In the cases when polyclonal anti-HMG1 antibody was used, it was added to the incubation mixture 20 min before the addition of DNA. After incubation for another 20 min the samples were resolved on 5% native polyacrylamide gel as described (*8*). On completion of electrophoresis, the gel was dried and exposed to either Amersham hyperfilm or a Molecular dynamics PhosphorImager screen. Quantification of band densities was performed using Image Quant Software. In some cases the autoradiographies were scanned with Gel-Pro Analyzer.

Determination of the Binding Affinity of HMG1 to DNA. Dissociation constants for binding of HMG1 to the different distorted DNAs were determined by evaluating the relative band intensities from EMSA. Since this assay revealed a single retarded band with all tested DNA probes within the range of protein concentrations used, 1 to 1 equilibria between free protein and the retarded oligonucleotide were assumed. Binding curves were built and used to determine the dissociation constants. The fraction of bound protein molecules (V) is related to the dissociation constant K_d by the formula $V = 1/(1 + K_d/[\mathrm{HMG1}])$. The values for both the K_d and the standard deviation were obtained by the least-squares best fitting using standard software.

Mass Spectrometry. Matrix-assisted laser desorption ionization-time-of-flight mass spectrometry (MALDI-ToF MS) was carried out with a Voyager-Elite XL Biospectrometry Workstation (Perseptive Biosystems, Framingham, MA), equipped with a 337 nm nitrogen laser. Mass spectra were recorded in the positive ion mode from 250 laser flashes with an accelerating voltage of 25 000 V in the linear mode, a 94.7% grid voltage, and a 150 ns pulse source delay time. The guide wire voltage was 0.15 and 0.30% for mass analysis and for tryptic digests of HMG1, respectively. The instrument was externally calibrated using human insulin or internally calibrated using auto-digested trypsin peptides. For mass analysis of HMG1, 3 μL of the sample were desalted by means of a ZipTipC4 pipet tip (Millipore) according to the protocol specified by the manufacturer. The elution was carried out with 10 μL of a 75% (v/v) acetonitrile aqueous solution containing 0.1% trifluoroacetic acid. A volume of 0.5 μL of each sample was mixed directly onto the target with 0.5 μL of a 10 mg/mL solution of sinapinic acid in a 30% (v/v) acetonitrile aqueous solution containing 0.1% trifluoroacetic acid. Sample spots were allowed to air-dry for a few minutes before inserting the target into the mass spectrometer. From the ZipTipC4-eluted fractions, 5 μL was lyophilized and then resuspended in 20 μL of 10 mM ammonium bicarbonate buffer, pH 7.8, containing 1 μg of

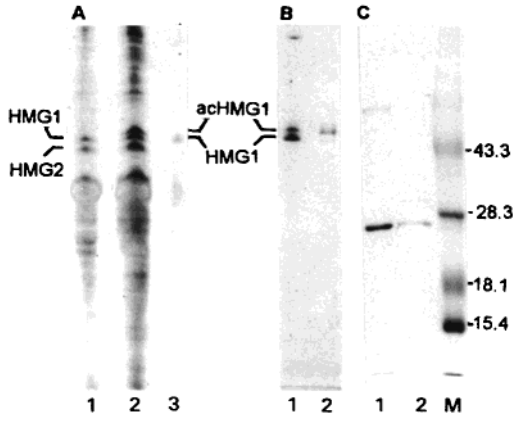

FIGURE 1: Electrophoretic characterization of HMG1 protein isolated from butyrate-treated Guerin ascites tumor cells. (A) Electrophoresis in 15% polyacrylamide-triton-urea gel of salt-extracted nonhistone proteins from unbutyrated (lane 1) and butyrated (lane 2) cells before column chromatography; lane 3, migration of purified parental HMG1. (B) Electrophoresis in 15% Triton-urea gel of HMG1 eluted from the first (lane 1) and second (lane 2) DEAE Sephacel column (see Experimental Procedures). (C) The same analysis as in panel B, but performed in SDS-containing gel; M, molecular mass marker proteins (kDa).

trypsin. Proteolysis was performed at 37 °C for 3 h. For mass spectrometric analysis of tryptic digests, 0.5 μL of tryptic sample was mixed directly onto the target with 0.5 μL of a saturated solution of 2,5-dihydroxybenzoic acid in a 50% (v/v) acetonitrile aqueous solution containing 0.1% trifluoroacetic acid.

RESULTS

Preparation of Acetylated HMG1 Protein. HMG1 from both butyrate-treated and untreated cells was extracted with salt under nondenaturing conditions and analyzed in Triton-urea gel. As Figure 1A shows, the zone of HMG proteins from butyrated cells contains an extra band immediately behind the band of HMG1 (lane 2), which is not present in the profile of the untreated cells (lane 1). Since acetylation reduces the positive charge of proteins, the observed extra band was attributed to acetylated HMG1 molecules. Such a conclusion was supported by the Triton-gel migration patterns of a mixture of acetylated and parental HMG1, eluted from the first DEAE-Sephacel column (Figure 1B, lane 1) and purified acetylated HMG1, eluted from the second DEAE-Sephacel column (Figure 1B, lane 2; see Experimental Procedures). When analyzed in SDS-gel, both samples migrated as a single band of identical molecular mass (Figure 1C, lanes 1 and 2). The identification of the extra band as HMG1 protein was confirmed immunochemically by the EMSA experiments, described below: the interaction of HMG1, either acetylated or nonmodified, with cisplatinated DNA and 4WJ demonstrated a super shift when performed in the presence of specific anti-HMG1 antibody (Figure 4, lanes 6, 7, 10, 11, and Figure 5, lane 4). When the same antibody was used in Western blot, it reacted with both acetylated and parental HMG1 (not shown). Finally, a mass spectral analysis was employed with the aim to determine the extent of acetylation and to identify the modified lysine-(s). Mass analysis of a sample eluted from the first DEAE-Sephacel column and containing both acetylated and unmodified HMG1 (see Figure 1B, lane 1) detected two

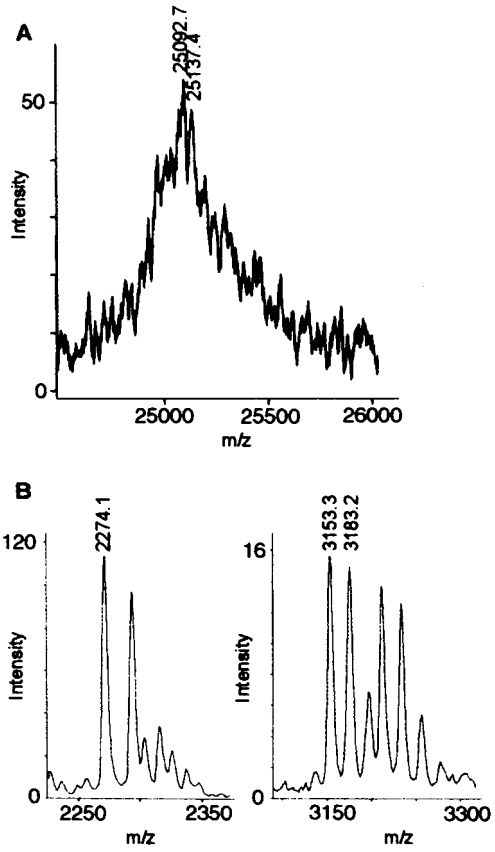

FIGURE 2: Mass spectrum identification of acetylated HMG1 protein. (A) Mass analysis of a sample containing both acetylated and nonmodified HMG1 (see Figure 1B, lane 1). The difference in the masses of the two peaks indicates that the acetylated protein contains a single acetyl group. (B) Mass analysis of purified and trypsinized acetylated HMG1. Note the presence of peaks at *m*/*z* 2274.1, 3183.2, and 3153.3 (see Results).

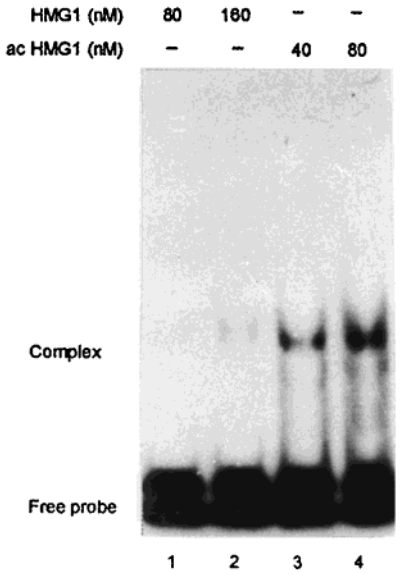

FIGURE 3: EMSA of the interaction of acetylated and nonmodified HMG1 with UV-irradiated 26 bp oligonucleotide, containing a single TT.

molecular species at *m*/*z* 25 093 ± 2 and 25 137 ± 2 (Figure 2A). Considering the range of precision for such molecular masses, the observed difference of 44 (the mass of the acetyl group is 42) indicates that under our conditions of in vivo acetylation in the presence of butyrate we have obtained a

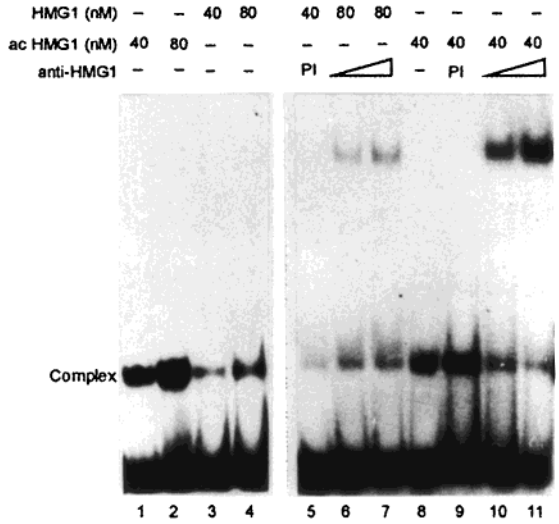

FIGURE 4: EMSA of the interaction of acetylated and parental HMG1 with site-specifically platinated 40 bp oligonucleotide. Binding reactions were performed in the absence (lanes 1−4) and in the presence (lanes 5−11) of anti-HMG1 antibody. PI, preimmune IgG.

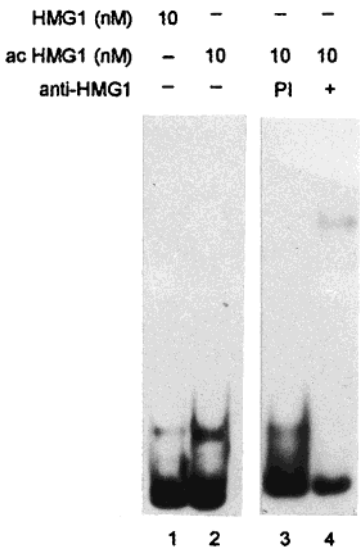

FIGURE 5: EMSA of the interaction of acetylated and nonmodified HMG1 with 4WJ. Binding reactions were carried out in the absence (lanes 1 and 2) and in the presence (lanes 3 and 4) of anti-HMG1 antibody. PI, preimmune IgG.

monoacetylated HMG1. The mass of the parental HMG1 showed some deviation from theoretically calculated value (24 762.5 with the N-terminal methionine absent). One reason could reside in the correlation theoretical mass/time-of-flight. While this should be perfect and linear, many factors such as posttranslational modifications and presence of ions other than H^+, e.g., Na^+, K^+, or even sinapinic acid adduct (delta mass +207/225) may lead to a substantial deviation (*35*). To avoid complications, the masses of both proteins were not assayed separately, but in a mixture. Having identified the two proteins as HMG1 by their electrophoretic and immunochemical properties, important in this case are not the absolute values for their masses, but the difference between them.

To identify the modified lysine, purified acetylated HMG1 and a control parental protein were digested with trypsin and subjected to mass analysis. The masses of the generated fragments (Figure 2B) were compared with their theoretical values using PEPTIDEMASS program (*36*). We searched for fragments involving lysine 2 or lysine 11 or both, the two residues shown to be acetylated upon using sodium butyrate (*27*). Informative in our case were the peptides containing residues 10−28 (M_R = 2276), 1−27 (M_R = 3111.5), and 3−29 (M_R = 3183.6). Fragments with *m*/*z* 2274.1 and 3183.2, both involving lysine 11 but lacking lysine 2 are present in the spectra of acetylated HMG1 (Figure 2B) and the parental molecule (not shown). This result indicates that lysine 11 is not acetylated. A peptide with mass 3111.5, containing both lysine 2 and lysine 11, which was observed in the spectrum of the nonmodified HMG1 (not shown), was not detected in the acetylated protein (Figure 2B). Instead, a peak at *m*/*z* 3153.3 was observed. Since the difference between the two peaks was 41.8, its presence was attributed to acetylation of lysine 2. Taken together these data show that the acetylated HMG1 is monoacetylated at lysine 2. This conclusion agrees with the earlier finding that upon butyrate-induced acetylation of HMG1, lysine 2 is more frequently modified than lysine 11 (ratio 5:1). The purified monoacetylated HMG1 was used in the DNA-binding experiments. The control, nonmodified protein used in these analyses migrated as a single band on both SDS and Triton-urea gels (not shown).

Binding of Acetylated HMG1 Protein to UV-Damaged DNA. The probe used was UV light irradiated 26 bp oligonucleotide, containing a single TT in the middle of the molecule. Previous experiments with this probe have shown that the cyclobutane pyrimidine dimer is the only product of irradiation and that it can be induced at a maximum yield not exceeding 5% of the adjacent thymines (*8*). Acetylated and nonmodified HMG1 proteins were reacted with the irradiated oligonucleotide and the specificity of the complex formation analyzed by EMSA. Figure 3 demonstrates the difference in the binding affinity of the two proteins: at a protein concentration of 80 nM, a band shift with the unmodified HMG1 is not visible, while the band obtained with the acetylated protein is rather intensive (compare lanes 1 and 4). Figure 3 shows also that the band shifted by the acetylated HMG1 migrates slightly faster than that of the parent molecule. One explanation might be a change in the conformation of the molecule as a result of the acetylation of the N-terminal region, change in the bending state of the complex, or both.

The dissociation constants derived from the EMSA-based binding curves (not shown) were 0.15 ± 0.02 μM for acetylated HMG1 and 1.68 ± 0.22 μM for the parental protein. As for the affinity of the two proteins to nonirradiated oligonucleotide (EMSA not shown), k_d for acetylated HMG1 was about twice lower than k_d for the parental protein (37.4 ± 4.6 and 84.6 ± 11.2 μM, respectively). These values are close to those previously reported for nonspecific binding of HMG1 to oligonucleotides (*8*).

Binding of Acetylated HMG1 Protein to Cisplatin-Damaged DNA. The interaction of HMG1, either acetylated or nonmodified, with site-specifically platinated 40 bp oligonucleotide was assayed by EMSA, and the results are presented in Figure 4. The acetylated HMG1 showed a higher affinity to the damaged probe than the nonmodified protein (compare lanes 1 and 2 with lanes 3 and 4, as well as lane 5 with lane 9). The use of specific polyclonal anti-HMG1

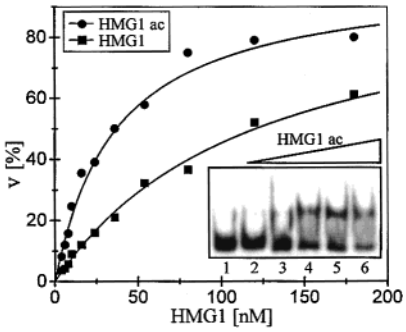

FIGURE 6: Binding curve of acetylated and nonmodified HMG1 on 4WJ. The relative band intensities of the complex formed are taken from EMSA. The insert shows gel retardation assay with acetylated protein (HMG1ac): lanes 1−6 correspond to protein concentrations of 2, 4, 8, 15, 25, and 35 nM; the rest of the data are from separate experiments. V is the fraction of bound protein molecules to a single 4WJ molecule (see also Experimental Procedures).

antibody reduced the intensity of the band shift with both acetylated and parental HMG1 and resulted in the formation of super shift. The dissociation constants for the acetylated and nonmodified HMG1, calculated from fitting curves (not shown), were 0.18 ± 0.02 and 1.17 ± 0.1 μM, respectively. The affinity of acetylated HMG1 to the control, nonplatinated probe was slightly higher than that of the parental protein (k_d values of 28.2 ± 3.9 and 52.9 ± 7.1 μM, respectively). The affinity constants for the interaction of HMG1 with both platinated and nondamaged control probe were close to those reported before by other authors (*6*).

Binding of Acetylated HMG1 to 4WJ. The band-shift assay (Figure 5, lanes 1 and 2) showed an increased affinity of acetylated HMG1 to synthetic 4WJ compared to that of the nonmodified protein. It should be mentioned, that unlike binding of HMG1 to cisplatin-treated and UV-damaged DNA, where a single band shift has been observed (*6*, *8*), interaction of HMG1 with 4WJ was reported to form two complexes with different binding affinity (*37*). In the experiments, with the 4WJ used, the binding affinities of acetylated and parental HMG1 were compared at protein concentrations generating a single band (Figure 5, lane 1 and 2). The obtained binding curve (Figure 6) confirms the mode of binding reported by the above cited authors at the same range of protein concentrations (*35*). The observed differences in k_d values (39 ± 16 and 122 ± 43 nM, respectively) were smaller than those found for UV- and cisplatin-damaged probes. The presence of antibody to HMG1 during incubation generated a super shift (Figure 5, lanes 3 and 4) as in the case with the cisplatin-treated oligonucleotide.

DISCUSSION

Mounting evidence demonstrates that transcription might be regulated by acetylation of nonhistone proteins (*38*). Considered together they show that this modification has divergent effects on DNA binding (*39*−*46*) and affects even binding to RNA (*47*). The consequence of acetylation of HMG1 and -2 and some HMG-box containing proteins remains to be established.

As yet, acetylated HMG1 has not been used in DNA-binding studies, although this modification of the protein has been known for a long time (*26*, *27*). Here we report for the first time the consequence of acetylation for the properties of HMG1 with an emphasis on the binding of the protein to its favorite target, the distorted DNA structures. Physiologically acetylated HMG1 was generated by growing cells in butyrate and the isolated protein was identified electrophoretically, immunochemically and by mass spectrometry as an HMG1 protein, monoacetylated at lysine 2 in the N-terminal region. We show that this modification increases the binding affinity of the protein to some distorted DNA structures. The difference relative to the parental molecule was most demonstrative with UV-irradiated oligonucleotides (about 15-fold), followed by cisplatin-damaged probes (about 6-fold) and less expressed with 4WJ.

The mechanism of the stronger binding of acetylated HMG1 to distorted DNA as compared to the parent protein is not clear. Since lysine 2, although in close proximity to the DNA-binding surface of HMG1 is not part of it (*4*), one explanation suggests an acetylation-induced conformational change of the molecule, allowing better access to the target as it was hypothesized for other nonhistone proteins (*39*, *42*). A possible mechanism involves acetylation-mediated changes in the interaction of HMG box with the C-terminal domain of the protein (*48*), shown to affect the DNA-binding properties of HMG1 (*14*, *18*−*22*). Whatever the mechanism of acetylation-induced changes in binding of HMG1 to DNA it will depend on the location of the modified lysine within the molecule (*38*). In this sense, our results on HMG1 extend the recent data on the acetylation of transcription factors p53, GATA1, EKLF, E2F1−3, and HMGI (Y). In the case of the first four proteins, the modified lysine lies adjacent to the DNA-binding domain and acetylation resulted in stimulation of DNA binding (*39*, *42*−*45*). As for HMGI (Y), the acetylated lysine falls within the DNA-binding domain and led to disruption of DNA binding (*41*). Regarding the acetylation of HMG-box proteins, two examples have been communicated so far, both referring to acetylation-affected interactions with other proteins. It was recently reported that the histone acetylatransferase CBP is recruited to and acetylates the ribosomal upstream binding factor (UBF, see ref *47*), involved in the ribosomal gene transcription. The observed significant activation of in vitro transcription from RNA polymerase I is supposed to result from acetylation-induced structural alterations in UBF (*49*). Earlier studies showed that acetylation of Drosophila TCF disrupted the binding of this transcription factor to its co-activator (*50*).

The biological role of the postsynthetic acetylation of HMG1 is difficult to predict merely because no specific functions have been firmly ascribed to this protein so far. It is generally regarded as an "architectural" element in the assembly of nucleoprotein complexes (reviewed in refs *3* and *51*), although a recent paper claimed that the protein is critical for proper transcriptional control by specific factors rather than for the organization of chromatin in the nucleus (*52*). Yet, taking into account that the sites for acetylation are conserved and considering the enhanced binding affinity of acetylated HMG1 to distorted DNA (the present work), this modification of HMG1 is by no means a fortuitous event, unrelated to its functions in the nucleus. The enhanced binding of HMG1 to damaged DNA as a consequence of its acetylation might be implicated in the repair mechanisms. Recently, the overexpression of HMG1 was shown to inhibit excision-repair of cisplatin-treated DNA, a finding that was

immediately related to the antitumor action of the drug (*53*). It is worth following the repair of such a DNA in the presence of acetylated HMG1. Furthermore, HMG1 and -2 were implicated in protein−protein interactions, shown to facilitate specific binding of regulatory proteins, such as steroid receptors, Hox proteins, p53, POU domain of Oct2 and human TATA box-binding protein to their target DNA sequences (*54*−*59*). The effect of acetylation on these properties of HMG1 is an intriguing extension of the present study.

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