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# Nucleosome Binding Properties and Co-Remodeling Activities of Native and in Vivo Acetylated HMGB-1 and HMGB-2 Proteins[†]

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ABSTRACT: The participation of HMGB-1 and -2 proteins in chromatin remodeling is investigated. Here, the ability of these proteins and their posttranslationally acetylated forms to affect SWI/SNF and RSC-dependent nucleosome mobilization was studied. Both proteins assisted nucleosome sliding induced by the two remodelers. Following acetylation, these proteins acquire the ability to bind to core particles, a property that has not yet been documented with parental proteins. We further report that compared to the nonmodified proteins, acetylated HMGB-1 and -2 exhibited both stronger binding to linker DNA-containing nucleosomes and a higher co-remodeling activity. Acetylation of HMGB-1 and -2 proteins enhanced binding of SWI/SNF to the nucleosome but did not affect its ATPase activity.

DNA in the eukaryotic nucleus is packed into a nucleoprotein complex termed chromatin. It exhibits a repeating structure with a basic unit, the nucleosome, consisting of an octamer of core histones (two each of H2A, H2B, H3, and H4), around which 1.75 turns of DNA are wrapped (*1*, *2*). All core histones contain a structured domain, the histone fold, and an unstructured $NH_2$ tail (*3*, *4*). The nucleosome impedes the access of protein factors to their cognate DNA sequence, thus creating an obstacle for numerous processes essential for the cell. The major strategies that the cell uses to overcome the nucleosome barrier are the posttranslational histone modifications, histone variants, and chromatin remodeling factors. The $NH_2$ termini of the core histones are required for the maintenance of the chromatin fiber (*5*) and mitotic chromosomes (*6*), and they are the main targets for posttranslational modifications (*7*, *8*). The posttranslationally modified histone $NH_2$ termini are specifically recognized by distinct protein complexes, and this recognition is an essential step in the functioning of these complexes (*8*). With regard to the nonallelic isoforms of conventional histones, the current view is that the incorporation of histone variants into the nucleosome results in a particle with modified structural and functional properties (*9*−*12*).

The chromatin remodeling factors (or remodelers) are multiprotein complexes, which are able, at the expense of the free energy liberated by the hydrolysis of ATP, to alter the histone−DNA interactions and mobilize the histone octamers (*13*−*15*). At least four different families of remodelers are described, namely, the SWI//SNF, ISWI, CHD, and INO80 families (*14*). The yeast RSC (remodels structure of chromatin) is a part of the SWI/SNF family (*16*). Both RSC and SWI/SNF exhibit a central cavity, the shape and dimensions of which suggest that they could bind a single nucleosome (*17*, *18*) and remodel one nucleosome at a time. Recently published electron cryomicroscopy data on the RSC−nucleosome complex demonstrate that indeed only a single nucleosome binds to the RSC cavity (*19*). Meanwhile, in light of the dominating idea that nucleosome sliding is initiated by a distortion of DNA into a loop at the nucleosome border (see ref *14*), attention was attracted by HMGB-1[1] and HMGB-2 proteins, abundant non-histone proteins in eukaryotic nuclei, that bind preferentially to distorted DNA (for reviews, see refs *20* and *21*). The highly dynamic manner of interaction with DNA and the induced transient bends upon binding are the "architectural" properties of these proteins, facilitating the formation of nucleoprotein complexes. Previous studies have demonstrated the ability of HMGB-1 to assist the ACF and CHRAC in inducing nucleosome sliding (*22*); i.e., it exhibited a co-remodeling activity. HMGB-1 was found to increase both the efficiency of binding of ACF to nucleosomal DNA and the extent of mobilization of the histone octamer at limited concentrations of the remodeler. On the other hand, we showed that in vivo acetylation of HMGB-1 at Lys2 strongly enhanced its binding to various forms of damaged DNA (*23*) and stimulated DNA end joining versus DNA bending (*24*). This motivated us to investigate the nucleosome binding properties and co-remodeling activities of native HMGB-1 and -2, both nonmodified and in vivo acetylated at Lys2. It is known that HMGB-1 binds to nucleosomes (*25*−*27*), but binding to core particles has not been documented so far. We show that at

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[1]Abbreviations: BSA, bovine serum albumin; DTT, dithiothreitol; PAGE, polyacrylamide gel electrophoresis; EMSA, electromobility shift assay; HMGB-1 and -2, high-mobility group B proteins 1 and 2, respectively; acHMGB-1 and -2, in vivo acetylated high-mobility group B proteins 1 and 2, respectively; PMSF, phenylmethanesulfonyl fluoride; TBE, Tris-borate-EDTA buffer.

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subpicomolar concentrations the acetylated HMGB-1 and -2, but not the nonmodified proteins, are capable of binding to both core particles and linker-containing nucleosomes. The nonmodified proteins assisted the SWI/SNF- and RSC-induced nucleosome sliding. Intriguingly, the acetylated forms of the proteins exhibited a higher co-remodeling activity.

## EXPERIMENTAL PROCEDURES

*Preparation of DNA*. The 255 and 241 bp DNA fragments, containing the 601-nucleosome positioning sequence at the middle or at the end of the fragment, were obtained by polymerase chain reaction (PCR) amplification from plasmids pGem-3Z-601 and p199-1, respectively (kindly provided by B. Bartholomew and J. Widom; see ref *28*). The 147 bp DNA fragment containing the 601-nucleosome positioning sequence was obtain by PCR amplification from plasmid pGem-3Z-601 by using primers 5′-CAGGATGTATATATCTGACACGTGCCT-3′ (forward) and 5′-CGAGAGAATCCCGGTGCCGAGGCC-3′ (reverse).

*Preparation of Proteins*. To prepare core histones, T7-based pET3a expression plasmids carrying the cDNA for *Xenopus laevis* full-length core histones H2A, H2B, H3, and H4 were propagated in *Escherichia coli* B121, expressed, and purified as described previously (*29*). HMGB-1 and -2 proteins, either unmodified or in vivo acetylated, were isolated by a nondenaturing salt extraction procedure from Guerin ascites tumor cells grown in the absence and presence of butyrate, respectively, as described previously (*23*). Yeast SWI/SNF complex was prepared following a published protocol (*30*).

*Nucleosome Reconstitution*. To reconstitute nucleosomes, an equimolar mixture of histones was dialyzed overnight at 4 °C against histone folding buffer [10 mM Tris (pH 7.5), 5 mM β-mercaptoethanol, and 1 mM EDTA] containing 2.0 M NaCl. The histones were then mixed at a 0.8:1 molar ratio with DNA consisting of $^{32}$P-labeled fragments (50 ng) and cold DNA of appropriate length, followed by a stepwise dialysis against a decreasing concentration of NaCl down to 10 mM (*31*).

*Electromobility Shift Assays (EMSAs) of HMGB-1 and -2 Proteins with Nucleosomes*. Fifty femtomoles of purified nucleosomes reconstituted from 147 bp (core particles), 241 bp (end-positioned), and 255 bp DNA (centrally positioned) were incubated with increasing amounts (as indicated) of HMGB-1 and -2 or their acetylated forms in 20 μL of 1× binding buffer [10 mM Tris (pH 7.5), 100 mM NaCl, 10 mM KCl, 1 mM EDTA, 1 mM dithiothreitol (DTT), 10% glycerol, and 0.2 mg/mL BSA], and 10 μg/mL sonicated calf thymus DNA. Reaction mixtures were incubated for 10 min at room temperature and then separated on 5% native polyacrylamide gels containing 0.5× Tris-borate-EDTA buffer (TBE). Protein:nucleosome molar ratios were 0.12, 0.25, 0.5, and 1 pmol for both nonmodified and acetylated HMGB-1 and -2 proteins.

*Micrococcal Nuclease Digestion*. Treatment of nucleosomes with micrococcal nuclease in the presence of HMGB-1 and -2 was performed as previously reported (*26*). Briefly, in a final volume of 30 μL, 250 fmol of middle positioned nucleosomes was incubated with 0.5 pmol of HMGB-2 or acHMGB-2 for 10 min at room temperature in 1× binding buffer [10 mM Tris (pH 7.5), 100 mM NaCl, 1 mM EDTA, 1 mM DTT, 10% glycerol, and 0.2 mg/mL bovine serum albumin (BSA)]. After 10 min, $CaCl_2$ was added to a final concentration of 1.5 mM, and the first "zero" sample of 5 μL was taken. Micrococcal nuclease (0.3 unit) was added to the rest of the reaction mixture, and digestion proceeded when a portion of 5 μL was taken at the indicated time points and added to 100 μL of EDTA (1 mM) and SDS (0.25%, w/v). The samples were phenol chloroform extracted, ethanol precipitated, and separated on a 12% native polyacrylamide gel. The dried gel was exposed on a PhosphorImager screen.

*Sliding of Nucleosomes*. The nucleosomes reconstituted with labeled DNA fragments (50 fmol) were preincubated for 5 min at room temperature with different HMGB-1 and -2 proteins (indicated in the figures legends) and then supplemented with SWI/SNF (1 μL) or RSC (1 μL) in remodeling buffer containing 10 mM Tris (pH 7.4), 5% glycerol, 100 μg/mL BSA, 1 mM DTT, 0.02% NP40, 40 mM NaCl, 2.5 mM $MgCl_2$, and 1 mM ATP. In all cases, the remodelers were used after prior titration for sliding activity. The reactions were stopped at the indicated time points by the addition of 1 μg of plasmid DNA, 0.02 unit of apyrase, and 10 mM EDTA. The reactions were visualized on a 5% polyacrylamide gel. Gels were dried, exposed on Phosphor-Imager screens, and quantified using ImageQuant (Molecular Dynamics).

*ATPase Activity Assay*. The ATPase activity of SWI/SNF in the presence HMGB-1 and -2 proteins was determined as previously described (*32*). Briefly, 100 ng of reconstituted nucleosomes was mixed with 1 μL of SWI/SNF and 1 μCi of [γ-$^{32}$P]ATP in a final volume of 8 μL [10 mM HEPES (pH 7.8), 50 mM KCl, 5 mM DTT, 0.5 mM PMSF (phenylmethanesulfonyl fluoride), 200 μg/mL BSA, 5% glycerol, and 3.5 mM $MgCl_2$]. Aliquots of 2 μL were taken at the time points indicated, and the reaction was stopped with 10 μL of gel loading buffer containing 90% formamide, 0.2% SDS, 10 mM EDTA, and dyes. ATP hydrolysis was analyzed on 15% denaturing polyacrylamide gels. Gels were dried and exposed to Phosphor-Imager screens.

*Binding of SWI/SNF to Nucleosomes*. The nucleosomes (100 fmol) were allowed to interact with SWI/SNF (1 μL) in the presence or absence of 1 mM ATP and 0.2 pmol of HMGB-1 and -2 proteins, either nonmodified or acetylated, for 20 min in remodeling buffer, and the complexes formed were visualized by an EMSA on a 4% polyacrylamide gel as described previously (*32*).

## RESULTS

Each experiment in the presence of HMGB proteins, either nonmodified or in vivo acetylated, was conducted with both HMGB-1 and HMGB-2. Since the data obtained were similar, the figures illustrate the results obtained with one of them.

*In Vivo Acetylation of HMGB-1 and -2 Proteins Enhances Its Affinity for Nucleosomes and Enables Binding to Core Particles*. Native HMGB-1 and -2, both nonmodified and acetylated, were isolated, purified, and characterized with regard to their binding to nucleosomes and co-remodeling activity. The proteins were purified nearly to homogeneity, since no additional bands were detected when they were run on either SDS or Triton-acid-urea gels (Figure 1A,B). Note that in agreement with previous data (*23*) the acetylated proteins exhibited a slower electrophoretic mobility in the Triton-acid-urea gel (in Figure 1B, compare lanes 1 and 2 and lanes 3 and 4).

Nucleosomes were reconstituted with recombinant core histones (Figure 1C) and $^{32}$P end-labeled DNA fragments: 255 and 241 bp for centrally positioned and end-positioned nucleosomes, respectively, and 147 bp for core particles. The binding of both nonmodified HMGB-1 and -2 and their acetylated forms in the

range of 0−1 pmol of protein to the three reconstituted nucleosome types was analyzed by EMSAs (Figure 2). In this concentration range, the nonmodified protein bound neither the linker-containing nucleosomes (Figure 2A,B, lanes 2−5) nor the core particles (Figure 2C, lanes 2 and 3). In contrast, the acetylated protein formed a complex with the core particles at 0.12 pmol (Figure 2C, lane 4) and with centrally positioned and end-positioned nucleosomes at 0.5 and 0.25 pmol, respectively (Figure 2A, lane 8, and Figure 2B, lane 7). Earlier reports have shown that in the concentration range of 5−10 pmol, the nonmodified HMGB-1 interacted with linker-containing nucleosomes, while binding to core particles has not been observed even at 50 pmol (*21*, *33*). We confirmed these results (not shown) and presented the first evidence that HMGB-1 could interact with core particles only if acetylated. We demonstrate also that the acetylated proteins bind more avidly to nucleosomes than parental molecules and that the interaction of acHMGB-1 or -2 with nucleosomes is independent of linker DNA.

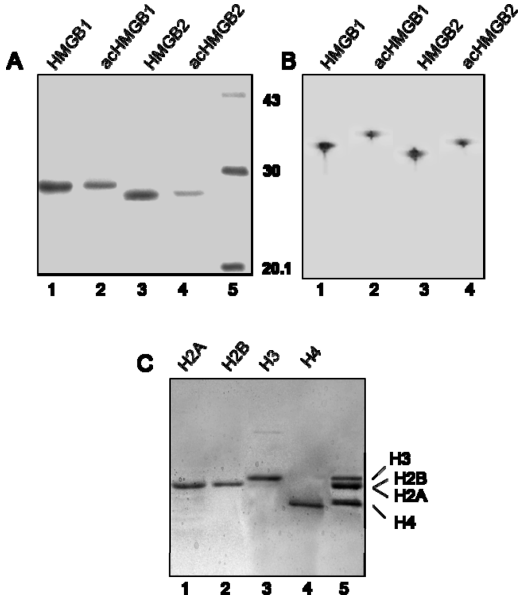


FIGURE 1: Electrophoretic analysis of native HMGB-1 and HMGB-2 and their in vivo acetylated forms (acHMGB-1 and acHMGB-2, respectively) used in this study: (A) 15% SDS−PAGE (lane 5, protein molecular mass markers with molecular masses indicated at the right), (B) 15% acetic acid-urea-Triton PAGE, and (C) 18% SDS gel of individual recombinant core histones used for nucleosome reconstitution (lane 5, mixture of core histones).

*Binding of Acetylated HMGB-1 and -2 to Centrally Positioned Nucleosomes Affects Their Accessibility to Micrococcal Nuclease*. Centrally positioned nucleosomes were reconstituted with $^{32}$P-labeled 255 bp 601 DNA fragment and incubated with 0.5 pmol of either nonmodified or acetylated HMGB-2. The mixture was supplemented with micrococcal nuclease, and the DNA fragments were separated via native 12% PAGE. As seen (Figure 3, lanes 6−9), the digestion pattern of nucleosomes in the absence of HMGB-2 is complex and comprises several bands, which are observed even after digestion for 1 min. A kinetic intermediate at ~150 bp (marked with an asterisk), corresponding to core particle DNA, is observed after digestion for 1 min (Figure 3, lane 6). Upon prolonged nuclease treatment, only subnucleosomal bands are detected (Figure 3, lane 9). The digestion profile of nucleosomes in the presence of nonmodified HMGB-2 was essentially the same (in Figure 3, compare lanes 7−9 with lanes 12−14) except that at the shortest digestion time (1 min) a slight protection from enzyme cleavage was observed in the presence of protein (in Figure 3, compare lanes 6 and 11). The presence of the acetylated HMGB-2, however, resulted in a completely different digestion pattern. Indeed, even after digestion for 4 min, the nucleosome was very weakly cleaved with some intact nucleosomes still present even after incubation with the enzyme for 20 min (in Figure 3, compare lane 19 to lanes 14 and 9). Moreover, at the longest time of digestion (20 min), the DNA fragments of ~150 bp were present in negligible amounts. We attributed this effect to the stronger binding of acHMGB-2 to the nucleosome, compared to the nonmodified protein, yet an alternative explanation might be that the nuclease activity per se is affected by the preparation of acetylated HMGB-2. In a control assay, the activity of the nuclease to 255 bp DNA was tested after prior incubation with acetylated HMGB2. The data obtained (Figure S2 of the Supporting Information) showed that the protein did not affect the enzyme activity, thus ruling out such a possibility.

*Acetylated HMGB-1 and -2 Exhibit Higher Co-Remodeling Activity Than Parental Proteins*. The data presented above suggest that acetylation of HMGB-1 and -2 creates new properties in these proteins, which is illustrated by their stronger binding to nucleosomes and the acquired ability to bind core particles. Do the acetylated HMGB-1 and -2 proteins acquire new functional properties? To test this, we studied their capacity to assist SWI/SNF nucleosome mobilization by comparing it with that of nonmodified proteins (Figure 4). Centrally positioned nucleosomes reconstituted on 255 bp 601 DNA were used

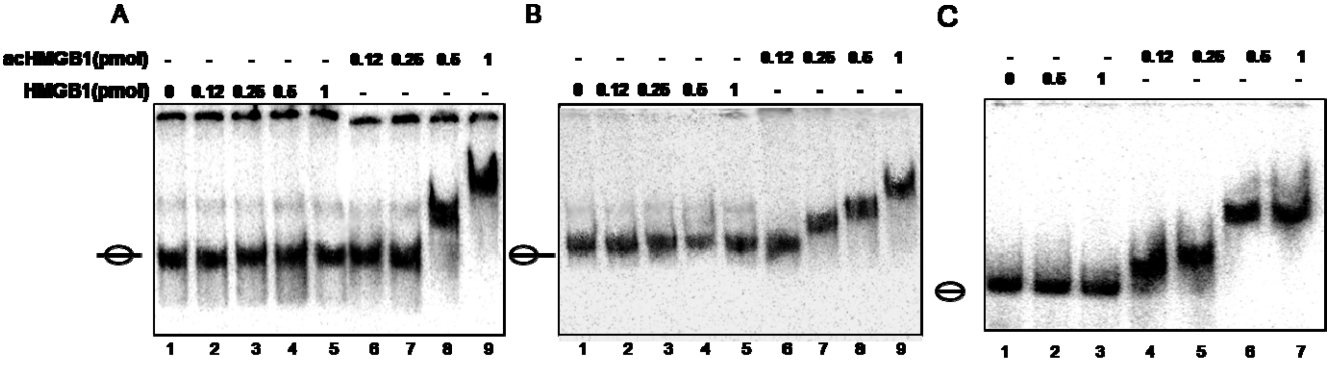


FIGURE 2: EMSA of the binding of HMGB-1 and its in vivo acetylated form (acHMGB-1) to linker-containing nucleosomes and core particles. (A) One hundred femtomoles of reconstituted centrally positioned nucleosomes was incubated with increasing amounts of HMGB-1 (lanes 2−5) or acHMGB-1 (lanes 6−9) both in the subpicomolar range. The position of the centrally positioned nucleosomes is indicated at the left. (B and C) Same as panel A but for end-positioned nucleosomes and core particles, respectively.

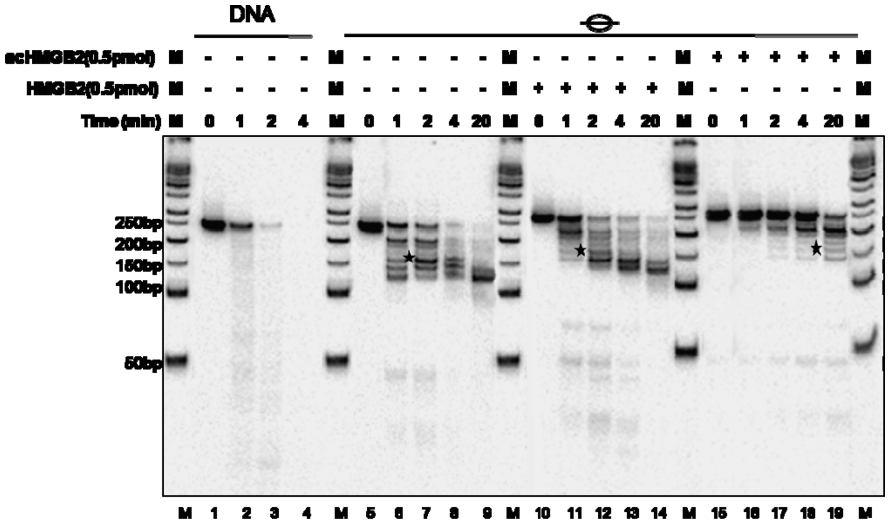


FIGURE 3: Effect of HMGB-2 and its in vivo acetylated form (acHMGB-2) on the accessibility of nucleosomes to micrococcal nuclease. Centrally positioned nucleosomes (250 fmol) were incubated for 10 min with 0.5 pmol of HMGB-2, either nonmodified or in vivo acetylated, and then digested with 12 units/mL micrococcal nuclease at room temperature for the times indicated (1−20 min). Upon completion of digestion, the DNA fragments were isolated and fractionated via 12% native PAGE. Lanes 1−4 and 5−9 show control experiments with micrococcal nuclease-treated naked DNA and nucleosomes, respectively. The asterisk marks the position of the 150 bp fragment. Lanes 10−14 and 15−19 show digestion of centrally positioned nucleosomes in the presence of HMGB-2 and acHMGB-2, respectively. M, markers for DNA length (in base pairs).

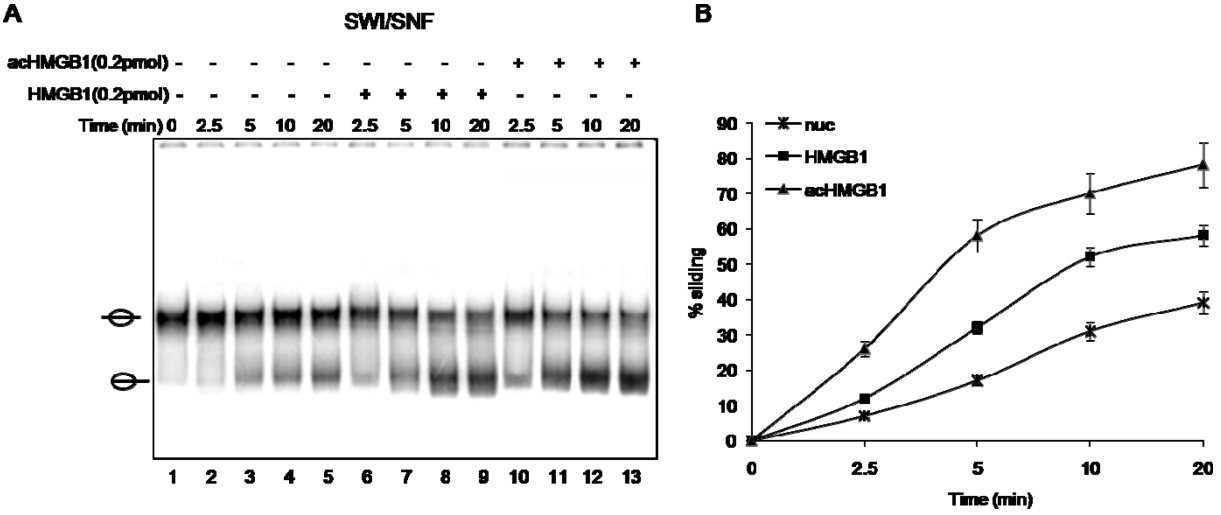


FIGURE 4: Co-remodeling activity of HMGB-1 and its in vivo acetylated form (acHMGB-1) in the presence of the remodeling complex SWI/SNF. (A) Fifty femtomoles of centrally positioned nucleosomes alone, or in the presence of 0.2 pmol of either HMGB-2 or acetylated HMGB-2 (acHMGB-2), was incubated with SWI/SNF (1 $\mu$L) at room temperature in the presence of ATP for the times indicated and run on a 6% native PAGE. The positions of the centrally positioned and end-positioned nucleosomes are indicated at the left. (B) Quantification of the data presented in panel A. The error bars are based on the data from three experiments, each conducted in duplicate.

in the assay. Incubation of the nucleosomes with SWI/SNF resulted in nucleosome sliding from the center of the DNA to its ends, the extent of which increased with time, and after incubation for 20 min, more than 35% of the nucleosomes were already end-positioned (Figure 4A, lane 5, and Figure 4B). The presence of the nonmodified HMGB-1 increased both the kinetics of nucleosome sliding and the total amount of end-positioned nucleosomes, reaching ~60% after incubation for 20 min (in Figure 4A, compare lanes 4 and 8 and lanes 5 and 9; see also Figure 4B). These effects were, however, definitely stronger with the acetylated HMGB-1 (in Figure 4 A, compare lanes 4 and 12 and lanes 5 and 13). Quantification of the data shows that the enhancement of the rate of nucleosome mobilization [the initial slope of the curves (Figure 4B)] compared to that in the absence of HMGB-1 was ~2- and ~4-fold for HMGB-1 and acHMGB-1, respectively. The fraction of nucleosomes that underwent sliding after incubation for 20 min increased from ~35% in the absence of HMGB-1 to ~60% in the presence of HMGB-1 and to ~80% in the presence of acetylated HMGB-1. The acetylation of HMGB-1 and -2 proteins, therefore, enhanced their capacity to assist SWI/SNF-mediated nucleosome sliding. It is worth mentioning that similar co-remodeling activities of HMGB-1 and -2, including the effect of acetylation, were observed with RSC, another remodeling complex (see Figure S1 of the Supporting Information).

The experiments described above have been performed with concentrations of HMGB-1 at which the protein does not form a stable complex with the nucleosomes. At higher protein concentrations (1 pmol), leading to formation of a stable complex, nucleosome sliding was inhibited (data not shown).

*Acetylation of HMGB-1 and -2 Proteins Enhances Binding of SWI/SNF to the Nucleosome but Does Not*

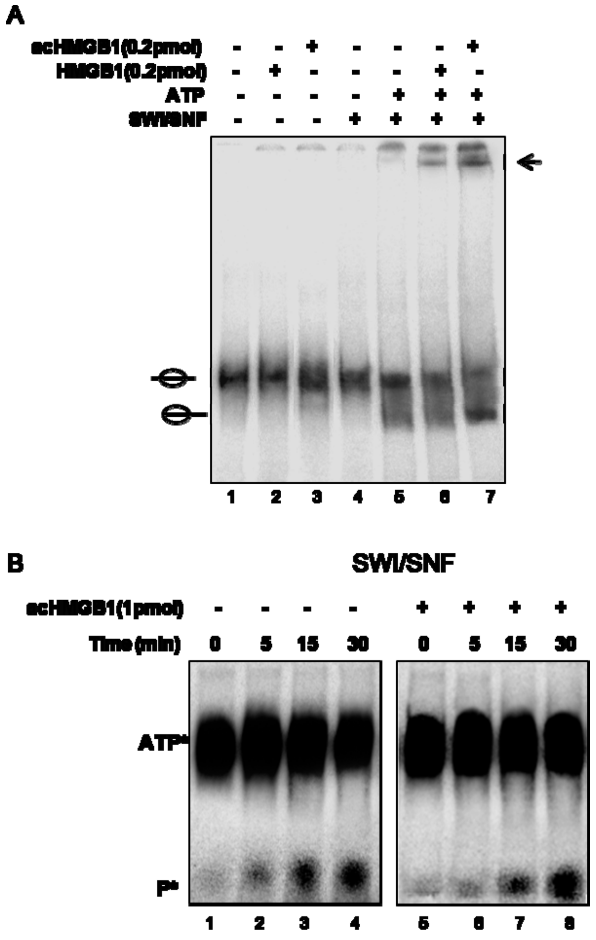


FIGURE 5: Effect of acetylated HMGB-1 on the binding of SWI/SNF to the nucleosome. (A) Centrally positioned nucleosomes (50 fmol) were incubated alone (lane 5) or in the presence of 0.2 pmol of either nonmodified HMGB-1 (lane 6) or acetylated HMGB-1 (lane 7) with SWI/SNF (1 $\mu$L) for 20 min at room temperature in the presence of 1 mM ATP. The products of the reaction were then separated via 5% PAGE under native conditions. The well-retained SWI/SNF−nucleosome complexes are marked with an arrow: lane 1, nucleosomes alone; lane 2, nucleosomes incubated with 0.2 pmol of nonmodified HMGB-1; lane 3, nucleosomes incubated with 0.2 pmol of acHMGB-1; lane 4, nucleosomes incubated with SWI/SNF in the absence of ATP. The positions of the nonmobilized and mobilized end-positioned nucleosomes are indicated. (B) Time course of SWI/SNF hydrolysis of ATP in the absence (lanes 1−4) or presence (lanes 5−8) of 1 pmol of acHMGB-1 analyzed via 15% PAGE.

*Affect Its ATPase Activity*. Next, we asked why the acetylated proteins exhibited stronger co-remodeling activity than their nonmodified counterparts. We favored two plausible explanations: (1) an enhancement of the ATPase activity of SWI/SNF and (2) its stronger binding to the nucleosome in the presence of the acetylated proteins, which led, in turn, to greater nucleosome mobilization. To study the efficiency of the remodeler's association with the nucleosomes, the SWI/SNF nucleosome sliding assay was conducted for 20 min in the presence of either nonmodified or acetylated protein followed by a separation of the reaction products on a native PAGE gel (Figure 5A). In agreement with earlier data, the presence of SWI/SNF retained some amount of nucleosomes in the wells [the large size of the SWI/SNF−nucleosome complexes does not allow their penetration in the gel (see ref *34*)]. HMGB-1 increased the amount of nucleosomes retained in the gel pocket, and this effect was stronger in the presence of acetylated protein (in Figure 5A, see lanes 5−7). Importantly, the presence of acHMGB-1 proteins does not increase the ATPase activity of SWI/SNF (in Figure 5B, compare lanes 1−4 with lanes 5−8).

## DISCUSSION

In this work, we have studied the behavior of the native HMGB-1 and -2 proteins, both nonmodified and posttranslationally acetylated, with regard to their involvement in the chromatin remodeling process. We show that acetylated HMGB-1 and -2, in contrast to parental proteins, bind in subpicomolar amounts to both core particles and centrally positioned and end-positioned nucleosomes. Earlier works have reported binding of recombinant HMGB-1 to linker-containing nucleosomes but not to core particles (*22*, *33*), and the binding was observed in the range of 5−20 pmol of protein. Here, we present the first evidence that upon acetylation HMGB-1 and -2 acquire the property to form a stable complex with the core particles on one hand and, on the other, to strongly enhance the affinity of the protein for linker-containing nucleosomes if compared to the unmodified protein. This is further confirmed by the observation that the binding of acHMGB-1 and -2 to nucleosomes protected their DNA upon digestion with micrococcal nuclease much more efficiently than the unmodified protein.

HMGB-1 and -2 were found to assist nucleosome mobilization induced by SWI/SNF. As documented above, such assistance we observed also with RSC, another remodeler belonging to the SWI/SNF family. A co-remodeling activity of HMGB-1 has already been demonstrated with remodelers from the ISWI family, namely, ACF, CHRAC, and ISWI (*22*). These data indicate that the boosting effect of HMGB-1 and -2 on nucleosome mobilization might be their general property, which is realized in a manner independent of the remodeler used. The effect of acetylated HMGB-1 and -2 on both the rate of nucleosome mobilization and the final amount of mobilized nucleosomes was stronger compared to that of parental proteins. It is worth mentioning that neither acHMGB-1 and -2 nor their parental forms affected the ATPase activity of SWI/SNF. Both proteins, however, enhanced the binding affinity of the remodeler for nucleosomes, the affinity being stronger in the presence of acHMGB-1 and -2 compared to that observed with the nonmodified proteins. Therefore, the stronger boosting effect of acetylated proteins on SWI/SNF nucleosome mobilization might be associated with the stronger binding of SWI/SNF to the nucleosomes in the presence of acetylated HMGB-1 and -2. Considering the transient interactions of HMGB-1 with nucleosomes on one hand and the higher affinity of remodelers for nucleosomal DNA in the presence of acHMGB-1 on the other, one may expect prolonged contacts between the remodeler and the nucleosomes in the presence of acetylated protein, compared to parental protein. On the bases of these data, we hypothesize a mechanism for a modulation of remodelers' activity by acetylated HMGB-1 and -2. Evidence that links histone acetyltransferases (HAT) to the remodeling process in vivo has accumulated (*35*−*37*). Since HAT may also acetylate non-histone proteins, including HMGB-1 (*38*, *39*), acHMGB-1 may well be generated during the remodeling process, and it could act at least in two ways. In light of the concept that HMGB-1 and -2 are key players in disrupting nucleosome structure (*40*), we may speculate that the more efficient binding of acetylated forms of these proteins to nucleosomes results in the formation of transiently perturbed particles that are better targets for the remodeling machines, and this in turn facilitates the sliding process. Another

possibility is suggested by the idea that acetylated lysines serve as signals that direct proteins to defined sites in chromatin rather than as simply charged molecules (*7*). Acetylated HMGB-1 and -2, together with the acetylated histones, may participate in creating modification patterns that affect gene regulation (*7*, *41*).

## ACKNOWLEDGMENT

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## SUPPORTING INFORMATION AVAILABLE

Effect of HMGB-2 on the RSC induced nucleosome sliding (Figure S1) and a control assay demonstrating that the acetylated HMGB-1 does not affect the activity of micrococcal nuclease (Figure S2). This material is available free of charge via the Internet at http://pubs.acs.org.

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