Distinct Domains in HMGB1 Are Involved in Specific Intramolecular and Nucleosomal Interactions

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ABSTRACT: HMGB1 is composed of two DNA-binding domains and a long acidic tail at the C-terminus. The acidic tail interacts with the DNA-binding domains of HMGB1 and with core histone H3 in the nucleosome. These interactions are important for modulation of the DNA and chromatin binding activities of HMGB1, as well as biological functions of HMGB1. However, the interactions are not fully characterized, because the tertiary structure of full-length HMGB1 is still unknown. Here we use chemical cross-linking, mass spectrometry, and epitope masking analysis to perform a detailed characterization of the inter- and intramolecular protein interactions of the acidic tail of HMGB1. We show that specific regions of the acidic tail participate in intramolecular interactions with Lys2 of HMGB1 and in intermolecular interactions with Lys36 and Lys37 of histone H3. The acidic tail is oriented by its location adjacent to the C-terminus of helix III of DNA-binding HMG box A in the HMGB1 molecule. These results suggest that the acidic tail modulates the biological functions of HMGB1 through these specific interactions.

High-mobility group box 1 (HMGB1)[1] protein is a multifunctional protein with activities both in the nucleus and in the extracellular milieu (*1*−*3*). HMGB1 is composed of two DNA-binding domains, named HMG box A and box B, and an acidic C-terminal tail (*3*). Each of the HMG boxes can bind to DNA without any DNA sequence specificity, preferentially to distorted DNA such as four-way junctions, semicatenated DNA, and bent DNA, and induce structural changes in the DNA (*4*−*9*). The acidic tail of HMGB1 is a continuous sequence of 30 acidic amino acids (*10*). Previous studies showed that the acidic tail has important roles in the functions of HMGB1. The acidic tail reduces the level of binding of HMGB1 to DNA (*9, 11, 12*), reduces the level of acetylation of the lysine 2 residues, and blocks the acetylation of the lysine 81 residue in HMGB1 by CBP (*13*). In cultured cells, the acidic tail is involved in transcriptional activation and affects the efficiency of V(D)J recombination (*14*−*16*). The acidic tail is required for ACF/CHRAC-mediated chromatin remodeling (*17*).

The tertiary structures of the HMG boxes have been well characterized (*18*−*20*). However, the structure of the complete HMGB1 molecule, including the conformation of the acidic tail, is still unknown because of its structural instability. Recent cross-linking experiments suggested that the acidic tail mainly interacts with the box B and linker regions (*21*). NMR studies of a synthetic peptide with the same amino acid sequence as the acidic tail of HMGB1 showed that the acidic tail interacts with specific amino acids in both box A and box B and suggested that these interactions shield the amino acid residues from other interactions (*22*). Recent NMR study by Watson et al. using a similar suite of truncation mutants of the acidic tail showed that the acidic tail interacts extensively with the DNA-binding surfaces of the HMG boxes (*23*). The acidic tail is also involved in the intermolecular interaction of HMGB1 with histone H3, whereby the C-terminal end of the acidic tail interacts with the N-terminal tail of histone H3. This interaction is required for the selective binding of HMGB1 to linker DNA between nucleosomes and is necessary for transcriptional activation (*15*). Therefore, the acidic tail is a functionally important region of the HMGB1 molecule involved in regulating the role of HMGB1 in chromatin.

Here we used a combination of chemical cross-linking, mass spectrometry, and epitope masking analysis to perform a detailed characterization of the intramolecular interactions between the acidic tail of HMGB1 and other regions of the molecule, and also of the intermolecular interactions of the acidic tail of HMGB1 with histone H3. We show that specific regions of the acidic tail of HMGB1 interact with two regions of HMGB1 and also with the N-terminal tail of histone H3.

EXPERIMENTAL PROCEDURES

Preparation of Proteins and Cross-Linking Experiment. Core histone octamer was purified from chicken erythrocyte nuclei as described previously (*24*). Purification of HMGB1, preparation of HMGB1 mutants (Figure 1A), and cross-linking experiments were performed as described previously (*15*).

Epitope Masking Assay by ELISA. HMGB1 or HMGB1 mutants were immobilized via a six-His tag at their N-termini

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[1] Abbreviations: HMGB1, high-mobility group box 1; EDC, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide; MALDI TOF-MS, matrix-assisted laser desorption ionization time-of-flight mass spectrometry; SDS−PAGE, sodium dodecyl sulfate−polyacrylamide gel electrophoresis; CBB, Coomassie Brilliant Blue.

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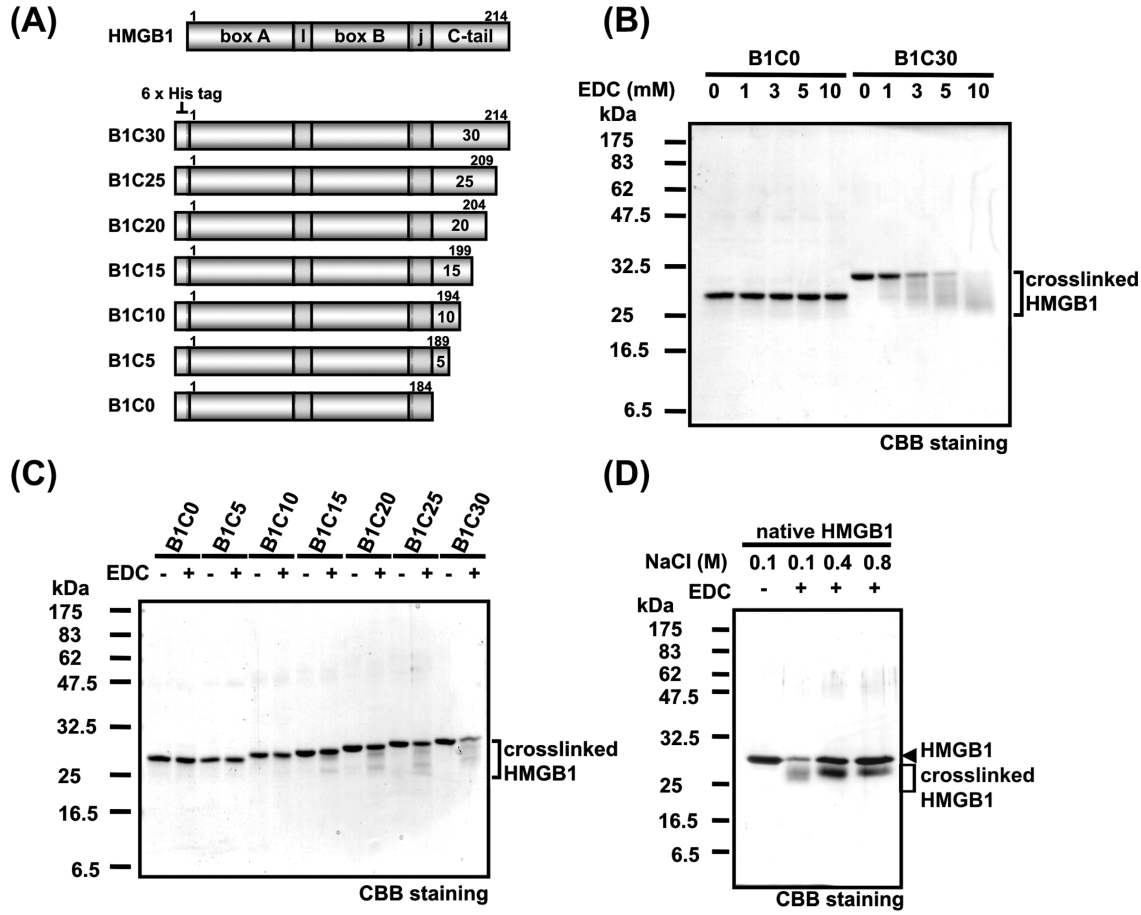

FIGURE 1: Identification of residues in the acidic tail involved in intramolecular interactions. (A) Schematic structures of HMGB1 and HMGB1 deletion mutants. (B) B1C0 and B1C30 were incubated with the indicated concentration of EDC in cross-linking buffer for 2 h, followed by SDS−PAGE analysis. Gels were stained with CBB. (C) Various acidic tail deletion mutants of HMGB1 (B1C0−B1C30) were incubated with 3 mM EDC in cross-linking buffer for 2 h, followed by SDS−PAGE analysis. Gels were stained with CBB. (D) HMGB1 was incubated with 3 mM EDC in cross-linking buffer containing various concentrations of NaCl for 2 h.

on a Ni-chelating 96-well plate (Qiagen) in PBS containing 1% (w/v) BSA and 0.1% (v/v) Triton X-100 for 2 h at room temperature. Unbound protein was removed from the plate, which was then washed three times with PBS containing 0.1% (v/v) Triton X-100. Mouse monoclonal antibodies KS1 (anti-HMGB1) or FBH7 (anti-HMGB1/2) (MBL) (*25*) were diluted in PBS-T [PBS containing 0.1% (v/v) Tween 20] and then added to the plate containing immobilized protein. After incubation for 1 h at room temperature, unbound primary antibody was washed out with PBS-T. Then, bound antibody was detected by alkaline phosphatase-labeled anti-mouse IgG (Chemicon) and *p*-nitrophenyl phosphate (Sigma) as the substrate.

Mass Spectrometric Analyses of Intra- and Intermolecular Cross-Linking Products. The intra- or intermolecular cross-linking was conducted in a low concentration of EDC (3 mM) to avoid excessive and nonspecific cross-linking reactions. The cross-linked products were separated by 12.5% SDS−PAGE, followed by CBB staining. Bands of interest corresponding to intra- or intermolecular cross-linking products of HMGB1 were cut out from gels and subjected to in-gel digestion with trypsin (Roche). After being concentrated and desalted with ZipTip C18 (Millipore), the peptides were analyzed in a Reflex III MALDI TOF-MS system (Bruker Daltonics) in positive linear mode. The mass spectra obtained were calibrated with Protein Calibration Standard I [from 3 to 20 kDa (Bruker Daltonics)] and analyzed with ProteinProspector (University of California, Los Angeles, CA) and Peptide Mass (ExPASy).

RESULTS

Identification of the Region in the Acidic Tail of HMGB1 Involved in Intramolecular Interactions by Cross-Linking with EDC. To identify the region in the acidic tail that interacts with other parts of HMGB1, we performed cross-linking studies with a series of HMGB1 mutants that have serial C-terminal five-amino acid deletions (Figure 1A).

The addition of increasing amounts of EDC to B1C30 (full-length HMGB1, residues 1−214) produced faster-migrating products on the SDS gel which represent intramolecular cross-linked products of HMGB1 as previously described (Figure 1B, lanes indicated by B1C30) (*21*), whereas the addition of EDC to B1C0 (from which the whole acidic C-terminal tail of HMGB1 has been deleted) did not produce such products. This indicates that the acidic tail of HMGB1 interacts with, and therefore was cross-linked to, other parts of HMGB1. Further cross-linking experiments using the series of deletion mutants in a low concentration of EDC (3 mM) showed that B1C15 (residues 1−199 of HMGB1) to B1C30 produced intramolecular cross-linked products, whereas B1C0 (residues 1−185) to B1C10 (residues 1−194) did not (Figure 1C). Thus, these results indicate that the region containing residues 195−199 of HMGB1 is involved in the intramolecular interaction.

EDC can cross-link the positively charged amino group of lysine or arginine and the negatively charged carboxyl group of glutamic or asparatic acid. Thus, we hypothesized that the intramolecular cross-linking between the acidic tail of HMGB1 and another region of HMGB1 results from an electrostatic interaction between an acidic amino acid in the acidic tail and a positively charged amino acid in another region of HMGB1. To confirm this possibility, a cross-linking experiment was conducted in the presence of increasing amounts of NaCl. Contrary to our expectation, the intramolecular cross-linking product of HMGB1 was clearly observed even at high NaCl concentrations (0.4 and 0.8 M) (Figure 1D). These results suggest that the intramolecular cross-linking within HMGB1 is not the result of an ionic interaction alone.

The Acidic Tail Is Cross-Linked with Lysine 2 of HMGB1. To identify the region of HMGB1 that forms intramolecular cross-links with residues 195−199 in the acidic tail, trypsin digestion peptides obtained from cross-linked native HMGB1 were analyzed by MALDI TOF-MS. The strategy for the characterization of the cross-linking region is shown in Figure 2A. The acidic tail is a continuous array of 30 glutamic acid and aspartic acid residues. Thus, the peptide derived from the acidic tail must be the largest peptide in trypsin-digested HMGB1. We compared mass differences between cross-linked and un-cross-linked HMGB1 acidic tail peptides to identify the protein fragment that is cross-linked to the acidic tail by EDC. MS analysis of the acidic tail peptides from un-cross-linked HMGB1 clearly showed three peaks, corresponding to the acidic tail and to the acidic tail with one or two lysine residues (Figure 2B, top panel; C-tail, K+C-tail, and KK+C-tail, respectively). EDC-cross-linked HMGB1 also yielded three peaks. The masses of these three peptides were each 601.3 Da larger than those arising from un-cross-linked HMGB1 (Figure 2B, bottom panel), indicating that the cross-linked acidic tail peptide contains an additional peptide with a molecular mass of 601.3 Da. A search for the 601.3 Da peptide among the predicted peptides one to six residues in length in a trypsin digest of HMGB1 yielded only one match (Figure 2C). These results indicate that the structural orientation of the acidic tail of HMGB1 is such that the region containing residues 195−199 interacts with lysine 2 of HMGB1.

Interaction between the Acidic Tail and the C-Terminal End of Helix III in HMG Box A Revealed by an Epitope Masking Assay. Several studies have proposed possible orientations of the acidic tail in the HMGB1 molecule (*21*−*23*), but the structure of the full-length HMGB1 molecule containing the acidic tail remains unknown. To further analyze the orientation of the acidic tail of HMGB1 under physiological conditions, we performed an epitope masking assay. The assay is based on the principle that if the acidic tail interferes with the binding of a monoclonal antibody to a known epitope, this interference should be due to steric hindrance of the epitope by the acidic tail. We used two monoclonal antibodies, FBH7 and KS1, which recognize S52-A53-K54-K56 and Y70-E71-R72 sequences in HMGB1 box A, respectively (*25*) (Figure 3A). We immobilized HMGB1 protein on a Ni-chelating 96-well plate to maintain the protein tertiary structure and then examined the effect of the acidic tail on the binding of these antibodies. Binding of FBH7 to cross-linked [B1C30 + EDC (Figure 3B, left panel)] and un-cross-linked [B1C30 (Figure 3B, left panel)] HMGB1 was similar. However, binding of KS1 to cross-linked HMGB1 [B1C30 + EDC (Figure 3B, right panel)] was weaker than that to un-cross-linked HMGB1 [B1C30 (Figure 3B, right panel)], suggesting that the acidic tail masked the KS1 epitope. This weak binding of KS1 to HMGB1 was not due to nonspecific modification of amino acids in the epitope by EDC, since treatment of B1C0 with EDC did not change binding of KS1 or FBH7 (data not shown). In further analysis with a series of acidic tail deletion mutants in this assay without EDC treatment, the mutants could be divided into two groups, B1C0 to B1C15 and

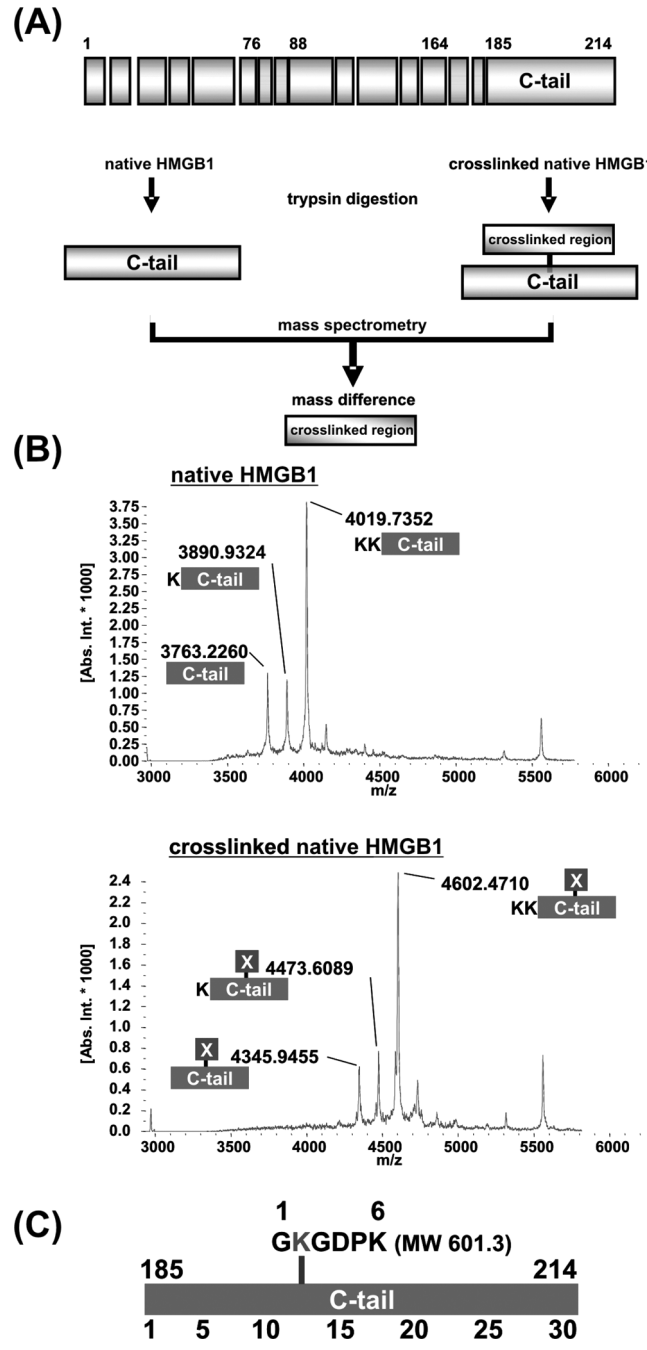

FIGURE 2: Identification of intramolecular cross-links formed by the acidic tail of HMGB1. (A) Schematic presentation of the experimental design. (B) Mass spectrum of the acidic tail derived from HMGB1 and cross-linked HMGB1 after treatment with trypsin. (C) Schematic representation of the intramolecular cross-linked product.

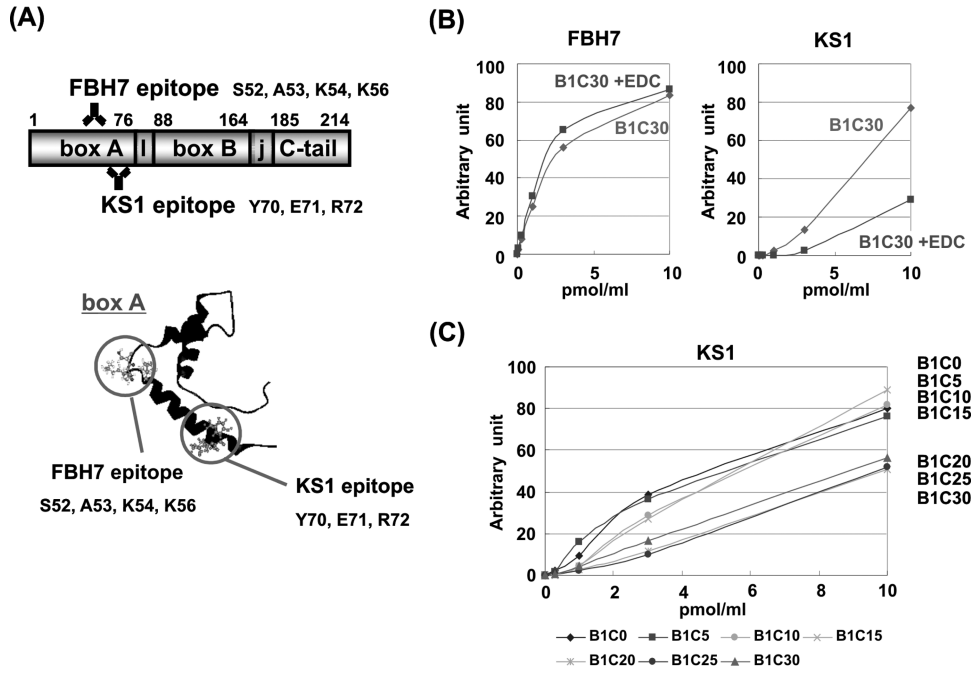

FIGURE 3: Epitope masking assay for examining intramolecular interactions of the acidic tail. (A) Anti-HMGB1 monoclonal antibody FBH7 recognizes an epitope containing S52, A53, K54, and K56 of HMGB1, and KS1 recognizes an epitope containing Y70, E71, and R72 of HMGB1. These epitopes are located in helix III of box A. (B) B1C30 or cross-linked B1C30 was immobilized on a Ni-chelating 96-well plate, and then the plates were incubated with either FBH7 or KS1. (C) Various HMGB1 mutants (B1C0 to B1C30) were immobilized and incubated with monoclonal antibody KS1.

B1C20 to B1C30, on the basis of their affinity for KS1, suggesting that the region of residues 200−204 in HMGB1 is involved in this interference (Figure 3C). These results suggest that the acidic tail of HMGB1, especially residues 200−204, masks the KS1 epitope located at the C-terminal end of helix III in HMG box A (Figure 3A).

Identification of the Region Involved in Intermolecular Interactions between HMGB1 and Histone H3. Our previous studies showed that the acidic tail of HMGB1 specifically interacts with the N-terminal tail of histone H3 in the nucleosome and the core histone octamer (*15*). To identify the sites of intermolecular interaction between HMGB1 and histone H3, cross-linking reactions and mass spectrometric analyses of cross-linking products were performed. As shown in Figure 4A, a cross-linking product between HMGB1 and histone H3 was prominent in reaction mixtures containing B1C30 and histone octamer but only weakly apparent in reaction mixtures containing B1C25 or B1C20 and histone octamer (Figure 4A). Other HMGB1 mutants yielded no intermolecular cross-linking product (Figure 4A). These results indicate that the five amino acids at the C-terminus of HMGB1, residues 210−214, make up the dominant cross-linking site with the N-terminal tail of histone H3.

To identify the site in histone H3 that interacts intermolecularly with the acidic tail of HMGB1, we used MALDI TOF-MS analysis to characterize peptides derived from the trypsin-digested HMGB1−histone H3 cross-linking product. The MS analysis gave three high-mass peaks which appeared to consist of a histone H3 peptide cross-linked with the acidic tail of HMGB1 [the mass of this peptide is 2868.6 Da without the acidic tail (Figure 4B)]. None of these peak masses corresponded to a peptide of histone H3 simply cross-linked with acidic tail peptide(s) of HMGB1. Therefore, we extended our analysis to include peptides originating from both intramolecular and intermolecular cross-links, since both intramolecular and intermolecular cross-linking products were detected in this reaction (Figure 4A). Finally, we could identify these products as containing histone H3 peptide 28−49 (the mass of this peptide is 2321.2 Da) cross-linked at Lys36 and Lys37 with the acidic tail (and +K, +KK) which itself has cross-links to residues 1−6 in HMGB1 [the mass of this peptide is 601.3 Da (Figure 4C)]. These analyses show that the acidic tail of HMGB1 binds to the N-terminal tail of histone H3 without disruption of the intramolecular interaction between the tail and the rest of HMGB1. This result is consistent with a recent NMR study that showed that residues 210−214 are potentially accessible for other protein partners (*23*).

DISCUSSION

These studies show that three distinct regions in the acidic tail of HMGB1 are involved in different protein−protein interactions. Residues 195−199 in HMGB1 interact with Lys2 of HMGB1. Residues 200−204 in HMGB1 are close to the KS1 epitope, which is located at the end of helix III in HMG box A (residues 70−72). In addition, the five C-terminal residues (residues 210−214) of HMGB1 interact with Lys36/37 of histone H3. These intra- and intermolecular interactions are summarized in Figure 4D. The neighboring regions in the acidic tail (residues 200−204 and residues 195−199) interact with the separate regions in the HMGB1 molecule (lysine 2 and residues 70−72, respectively). The tertiary structure of the HMG boxes reveals that the N-terminal tail and the end of helix III of HMG box A are adjacent (*18*−*20*). Thus, the neighboring regions in the acidic tail may cross over to interact with these two regions in HMG box A. Although we used HMGB1 and core histone octamer for this study, the results suggest that these interactions may determine the orientation of the long acidic tail of the

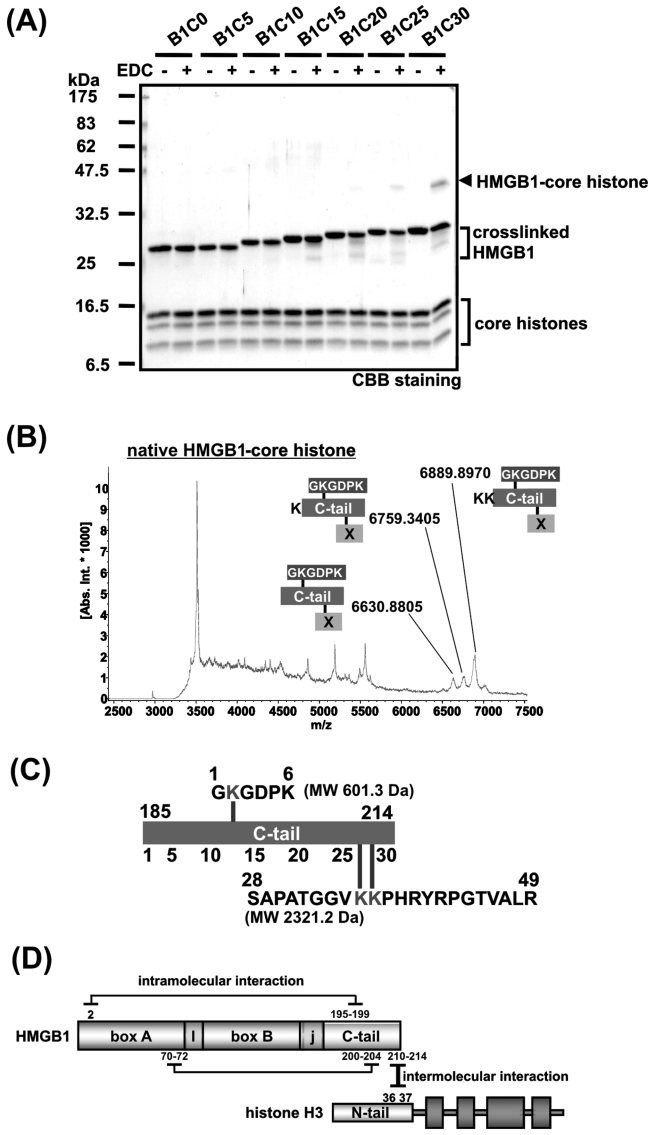

FIGURE 4: Identification of regions of interaction between the acidic tail of HMGB1 and histone H3. (A) Various HMGB1 deletion mutants and core histone octamer were incubated with or without 3 mM EDC for 2 h. The cross-linking products were separated via SDS−PAGE, and the gel was stained with CBB. (B) Mass spectrum of trypsin digests of cross-linking products. (C) Schematic representation of the intramolecular cross-linking product. (D) Summary of the multiple intermolecular and intramolecular interactions of the acidic tail of HMGB1.

HMGB1 molecule to optimize the interaction of the C-terminus of the acidic tail with the region containing Lys36 and Lys37 of histone H3 in the nucleosome core. In turn, this initial interaction of the C-terminal end of the acidic tail may orient the two HMG boxes on nucleosomal DNA (*15*).

Previous cross-linking experiment demonstrated that the acidic tail mainly interacts with HMG box B and basic linker regions rather than HMG box A (*21*). This finding is different from the results presented here, even though both experiments used HMGB1 and EDC as a cross-linking reagent. However, the experimental conditions, such as the salt concentration in the reaction mixture, are different. One factor that might account for this discrepancy is the difference in the salt concentration between the two experiments, since salt concentration affects the interaction of the acidic tail with the rest of HMGB1 (Figure 1D).

Recently, Watson et al. (*23*) used a similar suite of truncation mutants of the acidic tail together with NMR chemical shift perturbation mapping to show that the acidic tail interacts extensively with the DNA-binding surfaces of the HMG boxes. The results presented here provide additional information about the interaction of the acidic tail with HMG box A and the path of the acidic tail in HMGB1, since we showed that Lys2 of HMGB1 interacts directly with the region of residues 195−199 in HMGB1 by electrostatic binding. In addition, the epitope masking assay showed that the region of residues 200−204 is adjacent to the epitope of KS1, consisting of Tyr70, Glu71, and Arg72. These results give us new insight into the position of the acidic tail in the HMGB1 molecule and are consistent with the orientation suggested in the previous report (*23*).

The sequence of the C-terminal end of helix III in HMG box A is very close to the basic linker region, and it is easy to imagine that some region in the acidic tail, after crossing over the end of helix III, interacts with the linker region. Previous NMR and cross-linking studies of HMGB1 have shown that the acidic tail is close to the linker region (*13, 21, 22*). It is well-established that the acidic tail of HMGB1 negatively regulates its affinity for DNA and that the acidic tail also regulates the selective binding of HMGB1 to distorted DNA (*6*). HMGB1 binds to DNA via its two HMG boxes, but the affinities of these HMG boxes are not particularly strong. The affinity of HMGB1 for DNA is enhanced by the two basic linkers of HMGB1, located between box A and box B and between box B and the acidic tail (*9, 12*). These basic regions probably interact with the negatively charged DNA backbone, enhancing and stabilizing the binding of the HMG boxes to DNA (*9, 26*). It is possible that interaction between the acidic tail and HMG box A neutralizes the positive charge in the basic region of HMGB1, thereby weakening the binding of HMGB1 to DNA. Thus, the acidic tail of HMGB1 is not simply a continuous array of 30 acidic amino acid residues but plays important roles in intra- and intermolecular interactions with multiple regions of HMGB1 itself and with other molecules. These interactions of the acidic tail of HMGB1 probably modulate the functions of HMGB1 on, and its binding to, chromatin (*15, 17*). The acidic tail of HMGB1 is highly conserved, but not identical, among various mammalian species (*11, 14, 15*), suggesting that these acidic structures have similar but not identical functions depending on the exact structure of the array (*27*).

Surprisingly, intramolecular cross-linking of HMGB1 was detected in the presence of increasing concentrations of NaCl. EDC cross-links between directly bound positive and negative charges, and such binding should be broken by high NaCl concentrations. A recent study has suggested that the acidic tail interacts near the hydrophobic cores of HMGB1 and prevents denaturation of the molecule (*23*), and our observation also suggests that the acidic tail of HMGB1 is located inside of the hydrophobic cores.

Our previous report demonstrated that the five-amino acid sequence (DDDDE) at the C-terminus of the acidic tail (residues 210−214 in HMGB1) interacts with the N-terminal tail of histone H3, and that this interaction is critical for linker DNA-dependent binding of HMGB1 to the nucleosome and for transcriptional activation (*15*). The study presented here reveals that HMGB1 is specifically cross-linked with K36

and K37 of histone H3 (Figure 4). Histone H3, like other core histones, has many basic amino acids in its N-terminal tail. Although core histone octamer and HMGB1 were used as cross-linking substrates, the only intermolecular interaction we could detect was between the acidic tail of HMGB1 and specific basic amino acids in the N-terminal tail of histone H3. This specific interaction between HMGB1 and histone H3 may be induced by additional protein−protein interactions between HMGB1 and histone H3, since box A of HMGB1 interacts with histone H3 (*28*). However, the DDDDE sequence is well-conserved among higher eukaryotic HMGB1s. Thus, the DDDDE sequence itself may have specificity for the N-terminal tail of histone H3.

Lys2 of HMGB1 and K36 of histone H3 are modified by histone acetyl- and methyltransferases, respectively (*13, 29, 30*). The acidic tail of HMGB1 probably interacts with K36 of histone H3 by direct electrostatic interaction between positively and negatively charged amino acid residues (Figure 4C). The interaction may affect the levels of both post-translational modifications of the proteins. Likewise, the post-translational modification of histone H3 and HMGB1 may affect the intra- and intermolecular interactions of the acidic tail. Indeed, the acidic C-tail inhibits the acetylation of Lys2 of HMGB1 by CBP (*13*). Acetylation of Lys2 of HMGB1 modulates the DNA binding activity of HMGB1, and methylation of K36 of histone H3 is a marker of actively transcribing genes (*29, 30*). We propose that the acidic tail of HMGB1 participates in biological processes on chromatin by changing electrostatic interactions between proteins, thereby modulating their post-translational modifications and protein interactions.

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REFERENCES

1. Agresti, A., and Bianchi, M. E. (2003) HMGB proteins and gene expression. *Curr. Opin. Genet. Dev. 13*, 170–178.
2. Hock, R., Furusawa, T., Ueda, T., and Bustin, M. (2007) HMG chromosomal proteins in development and disease. *Trends Cell Biol. 17*, 72–79.
3. Lotze, M. T., and Tracey, K. J. (2005) High-mobility group box 1 protein (HMGB1): Nuclear weapon in the immune arsenal. *Nat. Rev. 5*, 331–342.
4. Saito, K., Kikuchi, T., and Yoshida, M. (1999) The mechanism of sequence non-specific DNA binding of HMG1/2-box B in HMG1 with DNA. *Protein Eng. 12*, 235–242.
5. Bustin, M. (1999) Regulation of DNA-dependent activities by the functional motifs of the high-mobility-group chromosomal proteins. *Mol. Cell. Biol. 19*, 5237–5246.
6. Thomas, J. O., and Travers, A. A. (2001) HMG1 and 2, and related 'architectural' DNA-binding proteins. *Trends Biochem. Sci. 26*, 167–174.
7. Gaillard, C., and Strauss, F. (2000) High affinity binding of proteins HMG1 and HMG2 to semicatenated DNA loops. *BMC Mol. Biol. 1*, 1.
8. Bianchi, M. E., Beltrame, M., and Paonessa, G. (1989) Specific recognition of cruciform DNA by nuclear protein HMG1. *Science 243*, 1056–1059.
9. Stros, M., Stokrova, J., and Thomas, J. O. (1994) DNA looping by the HMG-box domains of HMG1 and modulation of DNA binding by the acidic C-terminal domain. *Nucleic Acids Res. 22*, 1044–1051.
10. Tsuda, K., Kikuchi, M., Mori, K., Waga, S., and Yoshida, M. (1988) Primary structure of non-histone protein HMG1 revealed by the nucleotide sequence. *Biochemistry 27*, 6159–6163.
11. Bonaldi, T., Talamo, F., Scaffidi, P., Ferrera, D., Porto, A., Bachi, A., Rubartelli, A., Agresti, A., and Bianchi, M. E. (2003) Monocytic cells hyperacetylate chromatin protein HMGB1 to redirect it towards secretion. *EMBO J. 22*, 5551–5560.
12. Yamamoto, A., Ando, Y., Yoshioka, K., Saito, K., Tanabe, T., Shirakawa, H., and Yoshida, M. (1997) Difference in affinity for DNA between HMG proteins 1 and 2 determined by surface plasmon resonance measurements. *J. Biochem. 122*, 586–594.
13. Pasheva, E., Sarov, M., Bidjekov, K., Ugrinova, I., Sarg, B., Lindner, H., and Pashev, I. G. (2004) In vitro acetylation of HMGB-1 and -2 proteins by CBP: The role of the acidic tail. *Biochemistry 43*, 2935–2940.
14. Dai, Y., Wong, B., Yen, Y. M., Oettinger, M. A., Kwon, J., and Johnson, R. C. (2005) Determinants of HMGB proteins required to promote RAG1/2-recombination signal sequence complex assembly and catalysis during V(D)J recombination. *Mol. Cell. Biol. 25*, 4413–4425.
15. Ueda, T., Chou, H., Kawase, T., Shirakawa, H., and Yoshida, M. (2004) Acidic C-tail of HMGB1 is required for its target binding to nucleosome linker DNA and transcription stimulation. *Biochemistry 43*, 9901–9908.
16. Aizawa, S., Nishino, H., Saito, K., Kimura, K., Shirakawa, H., and Yoshida, M. (1994) Stimulation of transcription in cultured cells by high mobility group protein 1: Essential role of the acidic carboxyl-terminal region. *Biochemistry 33*, 14690–14695.
17. Bonaldi, T., Langst, G., Strohner, R., Becker, P. B., and Bianchi, M. E. (2002) The DNA chaperone HMGB1 facilitates ACF/CHRAC-dependent nucleosome sliding. *EMBO J. 21*, 6865–6873.
18. Hardman, C. H., Broadhurst, R. W., Raine, A. R., Grasser, K. D., Thomas, J. O., and Laue, E. D. (1995) Structure of the A-domain of HMG1 and its interaction with DNA as studied by heteronuclear three- and four-dimensional NMR spectroscopy. *Biochemistry 34*, 16596–16607.
19. Read, C. M., Cary, P. D., Crane-Robinson, C., Driscoll, P. C., and Norman, D. G. (1993) Solution structure of a DNA-binding domain from HMG1. *Nucleic Acids Res. 21*, 3427–3436.
20. Weir, H. M., Kraulis, P. J., Hill, C. S., Raine, A. R., Laue, E. D., and Thomas, J. O. (1993) Structure of the HMG box motif in the B-domain of HMG1. *EMBO J. 12*, 1311–1319.
21. Jung, Y., and Lippard, S. J. (2003) Nature of full-length HMGB1 binding to cisplatin-modified DNA. *Biochemistry 42*, 2664–2671.
22. Knapp, S., Muller, S., Digilio, G., Bonaldi, T., Bianchi, M. E., and Musco, G. (2004) The long acidic tail of high mobility group box 1 (HMGB1) protein forms an extended and flexible structure that interacts with specific residues within and between the HMG boxes. *Biochemistry 43*, 11992–11997.
23. Watson, M., Stott, K., and Thomas, J. O. (2007) Mapping intramolecular interactions between domains in HMGB1 using a tail-truncation approach. *J. Mol. Biol. 374*, 1286–1297.
24. Ausio, J., Dong, F., and van Holde, K. E. (1989) Use of selectively trypsinized nucleosome core particles to analyze the role of the histone "tails" in the stabilization of the nucleosome. *J. Mol. Biol. 206*, 451–463.
25. Ito, I., Mitsuoka, N., Sobajima, J., Uesugi, H., Ozaki, S., Ohya, K., and Yoshida, M. (2004) Conformational difference in HMGB1 proteins of human neutrophils and lymphocytes revealed by epitope mapping of a monoclonal antibody. *J. Biochem. 136*, 155–162.
26. Sheflin, L. G., Fucile, N. W., and Spaulding, S. W. (1993) The specific interactions of HMG 1 and 2 with negatively supercoiled DNA are modulated by their acidic C-terminal domains and involve cysteine residues in their HMG 1/2 boxes. *Biochemistry 32*, 3238–3248.
27. Lee, K. B., and Thomas, J. O. (2000) The effect of the acidic tail on the DNA-binding properties of the HMG1,2 class of proteins: Insights from tail switching and tail removal. *J. Mol. Biol. 304*, 135–149.
28. Stros, M. (1987) Binding of non-histone chromosomal protein HMG1 to histone H3 in nucleosomes detected by photochemical cross-linking. *Biochem. Biophys. Res. Commun. 147*, 301–308.
29. Kizer, K. O., Phatnani, H. P., Shibata, Y., Hall, H., Greenleaf, A. L., and Strahl, B. D. (2005) A novel domain in Set2 mediates RNA polymerase II interaction and couples histone H3 K36 methylation with transcript elongation. *Mol. Cell. Biol. 25*, 3305–3316.
30. Adhvaryu, K. K., Morris, S. A., Strahl, B. D., and Selker, E. U. (2005) Methylation of histone H3 lysine 36 is required for normal development in *Neurospora crassa*. *Eukaryotic Cell 4*, 1455–1464.

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