Published in final edited form as:

J Mol Biol. 2012 February 10; 416(1): 10–20. doi:10.1016/j.jmb.2011.12.004.

Basic N-terminus of yeast Nhp6A regulates the mechanism of its DNA flexibility enhancement

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Abstract

HMGB proteins are members of a class of small proteins that are ubiquitous in eukaryotic cells and non-specifically bind to DNA, inducing large-angle DNA bends, enhancing the flexibility of DNA, and likely facilitating numerous important biological interactions. To determine the nature of this behavior for different HMGB proteins, we used atomic force microscopy (AFM) to quantitatively characterize the bend angle distributions of DNA complexes with human HMGB2(Box A), yeast Nhp6A, and two chimeric mutants of these proteins. While all of the HMGB proteins bend DNA to preferred angles, Nhp6A promoted the formation of higher-order oligomer structures and induced a significantly broader distribution of angles, suggesting that the mechanism of Nhp6A is more like a flexible hinge than that of HMGB2(Box A). To determine the structural origins of this behavior, we used portions of the cationic N-terminus of Nhp6A to replace corresponding HMGB2(Box A) sequences. We found that the oligomerization and broader angle distribution correlated directly with the length of the N-terminus incorporated into the HMGB2(Box A) construct. Therefore, the basic N-terminus of Nhp6A is responsible for its ability to act as a flexible hinge and form high order structures.

Keywords

single molecule; DNA binding; DNA melting; HMGB2; atomic force microscopy

Introduction

HMGB proteins are members of a class of small proteins that are abundant in eukaryotic cells and sequence-nonspecifically bind to DNA^{1–3}. The defining feature of these proteins is that they contain one or more 80-residue “HMG box” domains. The Nhp6A protein from budding yeast^{4,5} contains a single HMG box, while human HMGB2 contains two HMG

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boxes³. These proteins are found in relatively high concentration in eukaryotic cells with a ratio of one HMGB protein per 5–10 nucleosomes in chromatin. Eukaryotes such as yeast and humans encode a variety of different HMGB proteins with different properties and abundances.

Among the striking properties of HMGB proteins is their ability to strongly bend DNA upon binding⁶. HMGB proteins appear to alter DNA structure through formation of hinges with enhanced flexibility^{7–9}. It is likely that all eukaryotes face comparable challenges in enhancing DNA flexibility, met to comparable extents by their respective complements of HMGB proteins. The exact biological functions of HMGB proteins are not yet clear. Current evidence suggests that HMGB proteins play multiple roles in chromatin structure and function¹⁰. Johnson and collaborators first found that HMGB proteins can replace prokaryotic HU proteins in the role of bending DNA¹¹. Research also has disclosed other important functions of HMGB proteins, such as enhancing transcription activation through facilitating transcription factor binding and function¹². For example, Nhp6A from budding yeast facilitates Gal4p binding to DNA⁵. HMGB proteins also participate in the facilitation of transcription elongation in yeast¹³. HMGB proteins play other important roles in enhancing protein-protein interactions by bending DNA^{14, 15}.

Although HMG “A box” and “B box” elements are similar in sequence and structure, properties of the “A box” domain of two-box HMGB1/2 proteins are surprisingly different from the “B box” domain¹. The A box domain differs slightly from the B box in terms of the shape and orientation of its first α -helix, and the identity of potential intercalating residues. Isolated A box domains reportedly bend DNA less⁹. The sequence of the his₆-tagged version of human HMGB2(Box A) illustrated in Fig. 1a highlights the alanine residue in its first α -helix that does not intercalate strongly¹. Furthermore, box A and box B domains show subtle differences in length and shape of the three α -helices¹. The single box HMGB protein Nhp6Ap is most similar to the box B family. Nhp6Ap is distinguished from HMGB2(box A) by the identity of the intercalating residue in its first α -helix (methionine in Nhp6Ap), a longer first α -helix, and a highly-cationic leader sequence.

To understand the role of the Nhp6A cationic N-terminal leader in its DNA bending ability, we probe the effect of a systematic increase in the size of the cationic leader in chimeric mutants. Recent research has shown that this cationic leader induces a surprisingly strong enhancement of protein function *in vitro* and *in vivo*⁶. The object of the present study is to obtain a more complete picture using the same proteins and Atomic Force Microscopy (AFM) methods.

Four HMGB proteins were therefore studied in this work: wild type human HMGB2(Box A), Nhp6A from budding yeast and chimeric mutants of HMGB2(Box A) with portions of the basic N-terminus of Nhp6A⁶, which we termed Mutant 1 and Mutant 2. All four proteins have sequences of approximately 100 amino acids (Fig. 1a). The principal difference between them is that Nhp6A contains a long cationic leader sequence of 16 amino acids, which is absent in HMGB2(Box A). Mutant 1 and Mutant 2 are chimeras having 5 and 9 amino acids, respectively, from the Nhp6A cationic leader sequence replacing HMGB2(Box A) residues (Fig. 1a). As shown in Fig. 1b, a common feature of HMGB group proteins is their L-shaped conformation determined by three α -helices (Fig. 1b). The L-shaped region interacts with the DNA minor groove to widen the groove and partially inserts one or more amino acid side chains between stacked base pairs³, thus bending DNA significantly. Fig. 1b shows molecular models of sequence-nonspecific architectural proteins mammalian HMGB2(Box A)¹⁶ and Nhp6A⁴. The long cationic leader sequence of Nhp6A (Fig. 1b, right, purple) is positioned in the DNA major groove, apparently asymmetrically neutralizing the negatively charged DNA phosphate backbone. Thus, DNA bending by

HMGB proteins may involve three kinds of interactions: minor groove widening, amino acid wedging, and asymmetric charge neutralization. Electrostatic interactions lead to the entropically-favorable release of cations from DNA as the dominant driver of HMGB nonspecific DNA binding and bending¹⁷. Entropically favorable dehydration of the macromolecular interface contributes to a lesser extent.

Understanding *how* these HMGB proteins enhance DNA flexibility is important to clarify their biological functions. Previous research results with HMGB proteins have suggested a mechanism based primarily on the formation of static DNA kinks^{2, 7}. Due to the sequence non-specific binding of HMGB protein, protein-induced DNA bends are difficult to probe structurally. While crystal structures of HMG-DNA complexes have been obtained^{18, 19}, in these structures two extra non-duplexed bases were incorporated, making an independent assessment of protein-induced bending impossible. Single-molecule techniques are well suited to resolve this issue. AFM and optical tweezers are two of these techniques. In this study we use AFM to characterize the bending of DNA by HMGB proteins at the single molecule level, comparing these results with observations from other experiments.

Results

Examples of AFM images of 4361-bp DNA in the absence of protein are shown in Fig. 2, while Fig. 3 shows typical images of DNA-protein complexes for all four proteins studied, when mixed at moderate protein to DNA base pair molar concentration ratios of 1:68 for HMGB2(Box A), 1:9 for Nhp6A, 1:37 for Mutant 1 and 1:50 for Mutant 2. A relatively higher protein concentration was used for Nhp6A because no binding was observed below that concentration. The proteins themselves are directly imaged, appearing as bright spots along the DNA molecules. Notably, HMGB2(Box A)-DNA complexes show small spots (Fig. 3a), while the proteins containing part or all of the Nhp6A cationic leader show much brighter and larger spots (Fig. 3b–e). These spots suggest protein oligomers bound together on the DNA. A variety of mechanisms could drive protein oligomerization^{20–22}. Both individual proteins and oligomers were observed to bind and bend DNA. These high resolution AFM images can be used to probe the relationship between protein oligomer size and DNA bending. If protein binding to DNA enhances the probability of further protein binding, the process is said to be cooperative²³. This phenomenon has been observed at HMGB2(Box A):bp ratios greater than 1:10, whereas the other proteins studied here exhibited cooperative binding at all concentrations studied^{23–26}.

Protein height distribution

To quantify the cooperative protein oligomerization observed in these experiments, we examined the protein height distribution, which should be directly related to the number of proteins bound at one location. Fig. 4a–d show protein height distributions for HMGB2(Box A), yeast Nhp6A, Mutant 1 and Mutant 2. A weighted moving average was generated and optimized at 7 or 9 points to smooth the discrete raw data. The distribution exhibits several strong peaks, which were fit with a sum of Gaussians (see Supplementary Data A) by minimizing $\chi^{221, 22}$. The individual peaks may represent individual protein layers, as the greater peak heights corresponded with larger oligomer sizes. In Supplementary Figure A2, we show that the volume of the aggregates on average increase with the cube of the height, as would be the case for a spherical or cubical shape. Therefore, the observed change in height directly reflects the increase in size of the protein oligomer at each site.

In addition to the observed oligomers, cooperatively bound regions of filamentous structure were observed for Nhp6A (Fig. 3e). From the height distributions, it is notable that for HMGB2(Box A) the average height distribution is lower than the other three proteins. This

suggests that the cationic leader, which is not present in HMGB2(Box A), may play a role in promoting protein oligomerization in the other three proteins.

Protein induced DNA bend angle distributions and analysis

An advantage of AFM is that the protein-induced DNA bend angle can be directly visualized and quantified^{27; 28}. Shown in Fig. 5 (a–d) are protein-induced DNA bend angle distributions for HMGB2(Box A), yeast Nhp6A, Mutant 1, and Mutant 2, respectively. Protein-induced DNA bend angles were measured directly at the protein binding site. The statistics are based on ~250 bend angles for each case. The bend angle distributions for HMGB2(Box A) and Nhp6A have means of $69.9 \pm 2.3^\circ$ and $59.5 \pm 2.5^\circ$, respectively, with standard deviations of 30.3° and 36.5° , respectively, where the uncertainty in mean angle is given by the standard error of measurement. For Mutant 1 and Mutant 2 we had to adjust the angle because of inactive protein bound (see Supplementary Data B). We found after angle adjustment that Mutant 1 and Mutant 2 induce mean bend angles of $57.8 \pm 2.4^\circ$ and $54.6 \pm 2.5^\circ$, respectively, with standard deviations of 30.3° and 34.2° , both very similar to Nhp6A. These results are summarized in Table 1. The bend angle distributions support a combined modified static kink and flexible hinge model for all proteins.

Using the protein height distributions from Fig. 4 we can study bend angle distributions for different oligomer sizes by setting certain height thresholds (See Supplementary data A for discussion). The average protein-induced DNA bend angle at different oligomer heights was determined and is shown in Fig. 6. We find that for Nhp6A, Mutant 1 and Mutant 2, the average bend angle increases as the oligomer size increases. However, for HMGB2(Box A), that relationship is reversed. The basis for this phenomenon is unknown, but the cationic leader clearly plays a role in determining this correlation, as the increase in bend angle with oligomer size is observed only for the proteins that contain a portion of the cationic leader. The fact that the effect of protein structures on DNA correlates with their size shows that they specifically interact with DNA and are therefore not simple aggregates, but instead cooperatively bound structures.

The standard deviation of bend angle measurements gives us information about additional induced flexibility at the protein binding sites and the possible influence of the N-terminal leader on this flexibility. Most of the width of the bend-angle distributions can be accounted for with a background of bare DNA flexibility (measured separately on random locations on bare DNA: $\sigma_{DNA} = 24^\circ$). The remaining bend-angle standard deviation is attributed to additional flexibility, induced at the protein binding site, which contributes σ'^2 to the bend-angle standard deviation. Thus the total Gaussian width, σ , of the distribution is equal to

$\sqrt{\sigma'^2 + \sigma_{DNA}^2}$. Fig. 7a shows a model distribution of bend angles, which is bi-Gaussian with peaks that are located at $\langle\beta\rangle$ and $-\langle\beta\rangle$ (Fig. 7b). In fact, the experimental angle measurement does not distinguish between positive and negative bends, and should produce a distribution similar to Fig. 7c, which shows the distribution of $|\beta|$. The measured distribution was then fit to this model (Fig. 5 (a–d)) and a χ^2 minimization was employed to find $\langle\beta\rangle$, σ and their errors. The results are summarized in Table 1. The best fit σ for the four types of HMGB proteins are plotted vs. the N-terminal leader size in Fig. 8a. There is clearly a strong dependence. Because σ depends on the length of the N-terminal leader and the protein oligomer size, in order to find the N-terminal leader induced bend-angle flexibility σ_N , the protein oligomer-size effect on bend-angle must be removed from σ . $\Delta\sigma_\beta$ is the contribution to bend angle variation due to the size effect and it is:

$$\Delta\sigma_\beta = \left| \left(\frac{d\langle\beta\rangle}{dh} \right) \sigma_h \right| \quad (1)$$

where $d\frac{\langle\beta\rangle}{dh}$ was obtained from the slope of Fig. 6 and σ_h is obtained from the oligomer height distributions (Fig. 4).

Then the N-terminal leader contribution to the variability is:

$$\sigma_N \approx \sqrt{\sigma_m^2 - \Delta\sigma_\beta^2} \quad (2)$$

The σ_N results are further summarized in Fig. 8b and Table 1. By comparing the four types of HMGB proteins, it is clear that the N-terminal leader increases the overall flexibility of the DNA at the protein binding site, resulting in complexes that more strongly resemble flexible hinges.

End-to-end distance method for measuring protein-induced DNA bending

We used a second method to estimate the average protein-induced DNA bend angle, which avoids possible errors in local bend angle measurements. This method is based on the Worm Like Chain (WLC) model^{29; 30} used by Rivetti^{31; 32} to model DNA deposited on a 2D mica surface. Rivetti first developed a modified WLC model for DNA bound with proteins³² in 3D. This modified WLC model was then adapted for protein DNA complexes that are deposited on mica surfaces and are able to reach a 2D equilibrium state [equation (3)]. This method requires measurement of the global protein-DNA complex end-to-end distance and the number of proteins bound for each individual DNA molecule.

$$\langle R_\beta^2 \rangle \approx 4pL \left(1 - \frac{2p\langle Np \rangle}{L} (1 - \langle \cos\beta \rangle)\right) \quad (3)$$

$\langle R_\beta^2 \rangle$ is DNA mean-squared end-to-end distance, p is the bare DNA persistence length, which is 57 ± 6 nm for 4361-bp pBR322 DNA^{7; 27}, L is the contour length of bare DNA, which is $1.48 \pm 0.11 \mu\text{m}$ ⁷, Np is the number of proteins bound to a single molecule and β is the protein induced DNA bend angle. The measured $\langle R_\beta^2 \rangle$ values for the four types of protein are $0.283 \pm 0.031 \mu\text{m}^2$ [HMGB2(Box A)], $0.291 \pm 0.040 \mu\text{m}^2$ (Mutant 1), $0.316 \pm 0.026 \mu\text{m}^2$ (Mutant 2), and $0.201 \pm 0.027 \mu\text{m}^2$ (Nhp6A). The Np parameter shows a near Poisson distribution with $\langle Np \rangle = 2.9 \pm 0.3$ and $\sigma_{Np} = 2.2$ [HMGB2(Box A)], $\langle Np \rangle = 4.0 \pm 0.2$ and $\sigma_{Np} = 1.2$ (Mutant 1), $\langle Np \rangle = 2.4 \pm 0.1$ and $\sigma_{Np} = 1.0$ (Mutant 2), and $\langle Np \rangle = 5.0 \pm 0.4$ and $\sigma_{Np} = 2.8$ (Nhp6A). Each estimate is based on measurements for 250–300 individual protein-DNA complexes. Using equation (3), $\langle \beta \rangle$ can be estimated by measuring $\langle R_\beta^2 \rangle$ and $\langle Np \rangle$. The results for $\langle \beta \rangle$ are summarized in Table 1.

For HMGB2(Box A), Mutant 1 and Mutant 2, the mean angle obtained by the two methods agrees within error. However for Nhp6A, the protein induced bend angle determined by direct measurement is $59.5 \pm 2.5^\circ$, with a standard deviation of 36.5° whereas the global method gives $92.8 \pm 1.7^\circ$. In addition to individual protein and protein oligomer binding to DNA, there also exists a filament structure that produces a rigid DNA segment. For Nhp6A, because protein binding is seen at higher protein:bp molar ratios (1:9) compared to the other three proteins, cooperative binding is enhanced. In fact, one or more small rigid segments can be seen on some Nhp6A images. Therefore, these small rigid segments must be taken into account in the model (Supplementary data C).

To account for this effect, an extra term:

$$4pl_f(\cos\beta_{f1}+\cos\beta_{f2}-1)+l_f^2 \quad (4)$$

must be added to the right side of equation (3), where p is the persistence length of bare DNA; l_f is the length of rigid segment; β_{f1} and β_{f2} are the two bend angles of the rigid segment with its two lengths of connecting DNA. Therefore, the Nhp6A protein induced bend angle deduced by mean squared end-to-end distance should be revised. We estimate that $\sim 1/4$ of Nhp6A is bound to DNA via cooperative binding. The average rigid fragment length was estimated to be $0.110 \pm 0.005 \mu\text{m}$ and the average β_{f1} and $\beta_{f2} \sim 73.8 \pm 10.6^\circ$, all of these lead to a calculated $\langle \beta \rangle$ of $81.8 \pm 5.2^\circ$, which is closer to the directly measured mean bend angle $59.5 \pm 2.5^\circ$ with a standard deviation of 36.5° . Understanding the remaining discrepancy will require a more sophisticated model.

Discussion

In this work, we compared two types of HMGB proteins, human HMGB2(Box A) and yeast Nhp6A, as well as two chimeric mutants. We quantitatively measured their abilities to bind and bend DNA, including the extent of cooperative binding, average bend angle, and bend angle distribution. We showed that the basic N-terminus of Nhp6A determines its ability to act as a flexible hinge when binding to and bending DNA.

Protein-induced DNA bend angles were directly measured from AFM images. Based on the bend angle distributions, HMGB2(Box A) induces larger average bend angles as compared with Nhp6A or the mutants. The two chimeric mutant forms of HMGB2(Box A), which contained a portion of the Nhp6A N-terminal cationic leader, induced bend angle distributions similar to those induced by Nhp6A. All bend-angle distributions support a static kink model with additional flexibility at the protein binding site. This flexibility appears to increase with the size of the cationic N-terminal leader, such that Nhp6A begins to resemble more of a flexible hinge. In addition, it is very interesting that for all but HMGB2(Box A), the larger oligomers have stronger DNA bending ability. The average protein-induced DNA bend angle was also estimated by global methods based on the mean-squared end-to-end distance and the number of proteins bound per DNA. The average DNA bend angles obtained by different experimental methods are compared in Table 1.

Cooperative protein oligomerization on DNA was observed and quantified in our experiments. From the height distributions, we determined the distribution of proteins bound at individual sites. It is notable that for HMGB2(Box A) the height distribution is weighted heavily towards lower heights, as compared with the other three proteins. The cationic leader of Nhp6A appears to confer a propensity toward formation of multimers. With higher protein concentrations we observed nearly saturated protein-DNA complexes. Cooperatively bound proteins also formed rigid structures. By comparing the AFM images for low and high protein concentrations, we saw a gradual transition in the number and size of protein oligomers binding to DNA. This coincides with the results from other single molecule experiments such as optical tweezers experiments (M. McCauley, L.J. Maher III, and M. C. Williams, in preparation),⁸ and magnetic tweezers experiments²⁶.

The quantitative AFM studies of DNA-protein complexes presented here provide significant new insight into the mechanism by which HMG proteins facilitate DNA bending. We show that yeast Nhp6A exhibits a very strong tendency to oligomerize upon binding to DNA, and the larger oligomers bend DNA with a much wider distribution of angles. By subtracting the oligomer size effect (Fig. 8b), we showed that this increased distribution width represents an actual increased flexibility of the DNA when bound by these structures rather than a heterogeneous distribution of static kinks at different sites. This result is consistent with a

primarily flexible hinge model for the binding mechanism of Nhp6A. The additional local flexibility of Nhp6A explains the biophysical basis for its higher effectiveness at promoting DNA cyclization compared to human HMGB2(Box A), a result that was initially surprising given that both proteins contain a single HMGB binding motif⁶. In contrast, human HMGB2(Box A) binds in much smaller domains with higher bend angles, while the larger domains tend to induce less DNA bending. Finally, by constructing chimeric mutants of HMGB2(Box A) that contain only a portion of the basic N-terminal leader of Nhp6A, we show that this domain is responsible for the increased oligomerization and increased flexibility of the protein-DNA complex, a result which is also consistent with earlier cyclization measurements for these proteins⁶. Importantly, because Mutant 1 and Mutant 2 share the poorly-intercalating alanine of the first α -helix with HMGB2(Box A), their Nhp6A-like DNA bending behavior points to the dominant role of the charged N-terminus (rather than side chain intercalation) in the added flexibility at the DNA bending site. These results suggest that the ability of Nhp6A to function as an autonomous enhancer of DNA flexibility can be attributed to stabilizing effects of the cationic N-terminus, and the ability of this segment to enhance oligomerization and a wider distribution of DNA bend angles. When isolated from its Box B partner in HMGB2, the Box A HMG domain lacks these properties. Thus, the basic N-terminus of Nhp6A differentiates it from Box A modules, and confers its ability to alter the flexibility of DNA, an ability that is key to the biological function of HMG proteins. These results will likely lead to further insights into the roles of these proteins in DNA replication and expression in eukaryotic cells.

Materials and Methods

Sample preparation

Isolated 4361-base pair (bp) plasmid pBR322 (Fermentas) was linearized by digestion with *Pvu*II (Fermentas) followed by phenol extraction. The DNA was diluted with 10 mM Tris-HCl or 10 mM HEPES (pH 8.0), 10 mM MgCl₂ to 231 nM (in base pair units) to avoid aggregation³³. HMGB proteins were purified as in⁸. Freshly cleaved mica was used as the substrate to deposit protein-DNA complexes. Because freshly cleaved mica is negatively charged, Mg²⁺ was used in the deposition buffer to bridge the binding between negative charged DNA backbone and the cleaved mica surface^{31; 34}. The initial protein solutions were: HMGB2(Box A): 376 nM, Nhp6A: 36 μ M, Mutant 1: 64 μ M, Mutant 2: 47 μ M, all in 20mM HEPES, pH 7.5, 5% glycerol, 100 mM KCL, 1 mM EDTA, and 1 mM dithiothreitol. The protein solutions were diluted 200–1000-fold with 10 mM Tris-HCl or 10 mM HEPES (pH 8.0), 10 mM MgCl₂. Diluted DNA and protein solution above were incubated for two min before deposition. 5 μ L of DNA-protein solution was deposited on freshly cleaved muscovite mica (Ted Pella Inc.) and allowed to equilibrate for 10 min^{27; 31}. The surface was rinsed with 5 ml distilled water and then air dried for 10–15 min, after which excess water was removed by careful blotting. Samples were then imaged immediately in air.

Experimental setup and data analysis

A Pico-Plus scanning probe microscope (SPM; Agilent Technology) was employed. The instrument was operated in tapping or intermittent contact mode in air. Nanosensors silicon supersharp AFM tips (SSS-NCHR) were employed (resonance frequency 190–330 kHz; spring constant 40 N/m). Tips typically have 2 nm radius of curvature, but an absorbed water layer limits lateral imaging resolution to 15 nm under the best conditions. The scan range was either 1 μ m $\times$ 1 μ m or 2 μ m $\times$ 2 μ m at 256 $\times$ 256 pixels. The scan rate was typically 4 lines per second. Both topography and phase images were analyzed. DNA contours were traced semi-automatically by using ImageJ software from NIH with the NeuronJ plug-in^{35; 36}. Proteins were identified and protein heights were measured by the Gwyddion software (Gwyddion.net) by carefully setting a height threshold at 0.7–1 nm. The

DNA bend angle was measured at the protein-binding site using Picoview SPM image analysis software from Agilent technology and using the traced contours to locate the entry and exit strands where DNA are bound by proteins. Since the position and orientation of these short segments of the contour relies on an optimized fit of the entire length of the DNA contour, the error in each angle measurement between these sections is reduced to about 8°, even with a pixel size of 7.8 nm for the lowest resolution 2 $\mu\text{m} \times 2 \mu\text{m}$ images⁷.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We thank Dattatri Nagesha and Emily Rueter for technical assistance and Sri Sridhar and the NU Nanomedicine IGERT for use of the AFM. The work was supported by NIH grants GM075965 (to LJM) and GM072462 (to MCW), as well as equipment supplement 3R01GM075965-04S1 supporting AFM instrumentation, NSF grant MCB 0744456 (to MCW), NSF grant DMR-1006007 (NEI), NIH and NCI IGERT grant number DGE-0504331, and the Mayo Clinic College of Medicine.

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(a)

HMGB2(Box A) MGSSH₆SSGLVPRGSHMLEDPGKGDPNKPRGKMSSYAFFVQTCREEHKKKHPDSSVNEAEFSKKCSERWKTMSAKEKSKFEDMAKSDKARYDREMKNYVPPKGDKKKKKDP

Mutant 1 MGSSH₆SSGLVPRGSHMLETRKKKDPNKPRGKMSSYAFFVQTCREEHKKKHPDSSVNEAEFSKKCSERWKTMSAKEKSKFEDMAKSDKARYDREMKNYVPPKGDKKKKKDP

Mutant 2 MGSSH₆SSGLVPRGSKKRTTRKKKDPNKPRGKMSSYAFFVQTCREEHKKKHPDSSVNEAEFSKKCSERWKTMSAKEKSKFEDMAKSDKARYDREMKNYVPPKGDKKKKKDP

Nhp6A MVTPRDPKKRTTRKKKDPNAPKRALSAYMFFANENRDIVRSWNP-D-ITEGQVGKKLGEKWKALTPEEKQPYEAKAQADKKRYESEKELYNATLA

(b)

Fig. 1.

Proteins analyzed in this work. (a) Sequence alignments of HMGB2(Box A), chimeric Mutant 1 and Mutant 2, and Yeast Nhp6A. Nhp6A and regions derived from its N-terminal basic leader are shown in bold. Amino acids that intercalate during DNA bending are underlined and shaded. (b) Molecular models of sequence-nonspecific architectural proteins: mammalian HMGB domain HMGB2(Box A) [left, PDB entry 1ckt (15)], and yeast HMGB protein Nhp6A [right, PDB entry 1j5n (16)]. DNA is colored cyan, and protein is shown in red. The cationic N-terminal leader of Nhp6A is in purple and intercalating amino acids are shown in green.

Fig. 2.
2 $\mu\text{m} \times 2 \mu\text{m}$ AFM image of bare, well-equilibrated PBR322 4361bp dsDNA.

Fig. 3.
a-d. 2 μm × 2 μm AFM image of Protein-DNA structures at low protein:bp ratios for HMGB2(Box A) (1:68), Nhp6A (1:9), Mutant 1 (1:37) and Mutant 2 (1:50) respectively. Fig 3e: 1 μm × 1 μm AFM image showing filamentous structure for Nhp6A at ×= 1:9.

Fig. 4. Protein height distributions for HMGB2(Box A) (a), Nhp6A (b), Mutant 1 (c) and Mutant 2 (d), respectively. A weighted moving average over 7 to 9 points (red lines) is employed to smooth the distributions.

Fig. 5. Protein induced DNA bend angle distributions for HMGB2(Box A), Nhp6A, Mutant 1 and Mutant 2 respectively and their best-fit σ of the proposed protein induced DNA bend angle model. The distributions are based on ~250 directly measured bend angles respectively.

Fig. 6.
Relation between protein height and mean bend-angle for HMGB2(Box A), Nhp6A, Mutant 1, and Mutant 2, respectively.

Fig. 7. Model for the bend-angle (β) distribution: (a) a static-kink of fixed angle is broadened by DNA flexibility into a Gaussian of width, σ . The kinks can bend in two directions in the plane producing a bi-Gaussian whose peaks are located at $\langle \beta \rangle$ and $-\langle \beta \rangle$. (b) The probability density function (PDF) function for β . (c) The PDF of experimentally measured angle, $|\beta|$, shows that $\langle |\beta| \rangle$ is slightly larger than $\langle \beta \rangle$.

Fig. 8. The relation between N-terminal leader length and (a) excess broadening of distribution, σ_m , and (b), portion of excess broadening attributable to binding-site flexibility, σ_N for HMGB2(Box A) (filled diamond), Mutant 1 (open square), Mutant 2 (open triangle), and Nhp6A (open circle). The solid line represents the measurement for DNA alone.

Table 1Average protein-induced DNA bend angle β and distribution variance σ .

Protein	HMGB2(Box A)	Mutant 1	Mutant 2	Nhp6A
Bend Angle (β)				
Model fit for AFM local bend angle $\beta^{(a)}$	64.5 $\pm$ 2.0 $^\circ$	45.5 $\pm$ 2.2 $^\circ$	37.5 $\pm$ 2.5 $^\circ$	54.5 $\pm$ 2.6 $^\circ$
AFM $\langle R^2 \rangle^{(b)}$	74.3 $\pm$ 5.5 $^\circ$	55.7 $\pm$ 21.8 $^\circ$	51.4 $\pm$ 25.2 $^\circ$	81.8 $\pm$ 5.2 $^\circ$
X ray Crystallography $^{(c)}$	$\sim$ 111 $^\circ$			
Distribution Width (σ)				
σ of local β distribution by AFM $^{(d)}$	26.0 $\pm$ 1.7 $^\circ$	28.0 $\pm$ 2.6 $^\circ$	34.0 $\pm$ 3.0 $^\circ$	38.0 $\pm$ 2.0 $^\circ$
N-terminal leader induced σ_N by AFM $^{(e)}$	25.0 $\pm$ 1.0 $^\circ$	25.6 $\pm$ 1.5 $^\circ$	33.4 $\pm$ 2.8 $^\circ$	37.8 $\pm$ 1.8 $^\circ$

 $^{(a)}$ Model fit of β to experimental distribution $^{(b)}$ $\langle R^2 \rangle$ is end-to-end distance method analyzed with Equation (3). $^{(c)}$ Crystallography data is from the *Drosophila melanogaster* single box HMGB protein HMG-D (protein data bank entry 1QRV). $^{(d)}$ σ of model-fit of β to experimental distribution $^{(e)}$ N-terminal-induced bend-angle flexibility from Equation (2) and Figure 8b.