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Understanding apparent DNA flexibility enhancement by HU and HMGB architectural proteins

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Abstract

Understanding and predicting the mechanical properties of protein/DNA complexes are challenging problems in biophysics. Certain architectural proteins bind DNA without sequence specificity and strongly distort the double helix. These proteins rapidly bind and unbind, seemingly enhancing the flexibility of DNA as measured by cyclization kinetics. The ability of architectural proteins to overcome DNA stiffness has important biological consequences, but the detailed mechanism of apparent DNA flexibility enhancement by these proteins has not been clear. Here we apply a novel Monte-Carlo approach that incorporates the precise effects of protein on DNA structure to interpret new experimental data for the bacterial histone-like HU protein and two eukaryotic high mobility group class B (HMGB) proteins binding to ~200-bp DNA molecules. These data (experimental measurement of protein-induced increase in DNA cyclization) are compared with simulated cyclization propensities to deduce the global structure and binding characteristics of the closed protein/DNA assemblies. The simulations account for all observed (chain-length and concentration-dependent) effects of protein on DNA behavior, including how the experimental cyclization maxima, observed at DNA lengths that are not an integral helical repeat, reflect the deformation of DNA by the architectural proteins and how random DNA binding by different proteins enhances DNA cyclization to different levels. This combination of experiment and simulation provides a powerful new approach to resolve a longstanding problem in the biophysics of protein/DNA interactions.

Introduction

Double-stranded DNA is intrinsically resistant to local structural deformations^{1–4}. This natural stiffness must be overcome in vivo^{5–8} so that very long genomes can fit within the prokaryotic nucleoid^{9–11} or the eukaryotic nucleus. A number of proteins known to severely

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Supporting information and methods are provided.

distort the double-helical structure contribute as well to the looping of DNA required for gene control and recombination^{7;12–14}. Such proteins seemingly enhance DNA chain flexibility^{6;15;16}.

Among these DNA-binding proteins are several “architectural” proteins (Fig. 1) that contribute to the sharp bending of DNA^{4;5;7;8;17–25}. For example, the abundant^{9;10} *Escherichia coli* histone-like protein from strain U93 (HU; Fig 1A) binds DNA as a homo- or heterodimer of 9.5-kDa subunits with little or no sequence specificity^{26–30}. HU binding induces a sizable DNA bend, appreciable untwisting, local dislocation, and hinge-like deformations of the helical axis^{29;31–39}. The degree of apparent bending extracted from X-ray crystallographic, biochemical and FRET experiments varies from 65° to ~150°, with most reported values at the higher end of the range. HU appears to directly facilitate DNA loop formation during repression of the *E. coli gal* operon^{40–42}. An A/T-rich sequence element apparently acts as the preferred HU binding site in the *gal* DNA loop. A direct Gal repressor-HU interaction has been proposed. Although HU had not previously been implicated in facilitating repression by DNA looping in the *lac* operon, our recent studies^{17–19} have shown that *lac* repression weakens in the absence of HU. Earlier work showed that the loss of HU causes nucleoid instability and growth defects in *E. coli*^{27;33;43}.

The abundant, sequence non-specific high mobility group B (HMGB) proteins in eukaryotes appear to perform roles analogous to prokaryotic HU^{8;44}. HMGB proteins contain one (Fig. 1B) or two (Fig. 1C) ~80-amino acid domains that fold into “L”-shaped structures composed of three alpha helices^{45–53}. The *Saccharomyces cerevisiae* Nhp6A protein (Fig. 1B) contains a single B-like HMG box. The *Rattus norvegicus* HMGB1 protein (modeled in Fig. 1C) contains similar HMG boxes “A” and “B.” Unstructured charged domains are also observed in HMGB proteins (Fig. 1D). HMGB proteins induce significant DNA bending through van der Waals and electrostatic interactions of the globular domains with the minor groove. The bending occurs in concert with significant changes in the twisting and lateral displacement of adjacent base pairs. Amino acid intercalation may enhance the local distortions⁵⁴. Thermodynamic⁵⁵ and FRET spectroscopy studies^{55;56} of the binding of an individual HMG box (B) from HMGB1 to DNA report an equilibrium dissociation constant of ~10 nM and a 40° induced bend angle. NMR measurements of the single-box Nhp6A protein from yeast reveal an overall DNA bend of ~70°⁵⁰. X-ray crystallography suggests an induced DNA bend angle of 111° for HMG-D⁵⁷, and 61° for the complex of HMGB1(box A) with cis-platin-modified DNA⁵¹. DNA bending by a designed protein containing HMG boxes from SRY and HMGB1 was found to be 101° in a detailed NMR analysis⁵². Finally, studies using single molecule force spectroscopy²¹ have estimated DNA bending at 99° for the isolated box A of mammalian HMGB2 protein, and 77° for mammalian HMGB1 containing both boxes A and B.

Low concentrations of HU enhance the deformability of DNA, while high protein concentrations appear to stiffen the chain (with the apparent persistence length, a value deduced by fitting the experimental data to the expected behavior of an ideal wormlike representation of DNA⁵⁸, dropping from 55 nm to 15 nm at 40 nM HU but increasing to 146 nm at 800 nM HU^{38;59}). The binding of HU and HMGB proteins also perturbs the force-extension properties of single DNA molecules^{20;21;36;60}, reducing the force on DNA at a given imposed extension and increasing the length of DNA at the overstretching transition compared to a protein-free duplex. Interpretation of these measurements in terms of a wormlike chain model of DNA suggests that human HMGB2 box A enhances the bending flexibility of DNA, i.e., reduces the apparent persistence length from 50 nm (~150 bp) to 10 nm (~30 bp) at 100 mM salt, and to 5 nm (~15 bp) at 50 mM salt. At saturation, the binding of this HMGB protein increases the DNA contour length by 16 percent²⁰. The binding site of the protein is estimated to be 5–6 bp, and the K_d to be ~50–100 nM, depending on salt

concentration. If it is further assumed that the protein bends a wormlike DNA in a fixed direction, the average bend angle induced by HMGB2 box A is estimated to be 114° at 50 mM and 87° at 100 mM monovalent salt. A more recent estimate of the DNA bending angle, from a similar analysis of the same type of optical-tweezers experiments, is 99° for this protein domain²¹. Interestingly, two-box HMGB proteins may bind DNA with the protein domains out of alignment⁵², such that the NMR structure of an artificial protein of this type shows a DNA bend angle of 77° , and interpretation of single-molecule experiments gives a comparable value²¹.

The central hypothesis in this work is that an appropriate, structure-based computational model can clarify how the binding of architectural proteins such as HU and HMGB proteins at non-saturating concentrations enhances the likelihood that DNA chain termini come into close contact compared to the ends of a naked duplex. Each randomly-bound architectural protein induces a directed (anisotropic) deformation or point of DNA flexure. While HMGB proteins bind tightly to unusual DNA structures such as cruciforms, binding to B-form DNA is transient for low concentrations of HMGB proteins^{31;61–64}. This implies that DNA/architectural protein complexes exist as an ensemble of rapidly-interconverting species with the bound DNA sampling a large number of compact spatial forms (Fig. 2). The end-to-end distances of most of these configurations should be shorter than the corresponding distances of protein-free DNA molecules. Thus, HU and HMGB proteins should accelerate ligase-mediated DNA cyclization (Fig. 2). Given the complex distortions of DNA seen in high-resolution HU- and HMGB-bound structures, this effective DNA compaction may not necessarily translate to a simple modification of DNA wormlike chain behavior, such as a decrease in the apparent persistence length.

While previous experiments have demonstrated the ability of HU and certain HMGB proteins to support ligation of DNA into small circles^{7;22;25;65}, it is strictly more precise and quantitative to measure the *j*-factor (relative concentration of one DNA terminus in the vicinity of the other) to distinguish any enhancement of end joining, *per se*, from effects of protein binding. For this purpose we have extended the method described by Vologodskaya and Vologodskii⁶⁶, deriving a model based on ordinary differential equations to fit kinetic data from ligase-catalyzed cyclization reactions⁶⁷.

Our recent Monte-Carlo simulations of HU-bound DNA⁶⁸ reveal the importance of accounting precisely for the distortions of double-helical structure imparted by protein in deciphering the cyclization properties of DNA. A wormlike chain representation of DNA fails to account for the full chain-length dependence of the *j*-factors of duplexes that incorporate the complex structural arrangements observed in high-resolution HU-DNA complexes. The classical depiction of DNA in terms of three parameters — the persistence length, the twisting modulus, and the double-helical repeat⁵⁸ — assumes a much more limited range of DNA deformation than that induced by HU^{29;37} and does not consider the unexpected chain-length-dependent uptake of protein on DNA found in the simulations⁶⁸. The present work gives us the opportunity to compare the observed *j*-factors of HU- and HMGB-bound DNA molecules with the predicted values for chains that are randomly decorated and realistically deformed by HU or by one- or two-box HMGB proteins. The dual approach also allows us to decipher the roles played by different nonspecific binding proteins in both the enhancement of ring closure and the organization of overall DNA folding.

Results and Discussion

Affinity and cooperativity of architectural protein binding to DNA

Previous studies have explored and modeled features of HU/DNA interactions^{68–71}. We therefore began by determining the apparent equilibrium binding affinity of HMGB proteins Nhp6A and HMGB1(A+B) for a 20-bp DNA duplex where it is assumed that, at most, one HMGB protein could bind the short DNA probe. The sequence was chosen to be ~50% G/C and lack sequence motifs known to cause intrinsic curvature, allowing for binding affinity estimates for these sequence-nonspecific proteins. Because transient binding limits applicability of electrophoretic gel mobility shift assays⁶⁴, affinity was assayed by fluorescence anisotropy measurements. Representative results are shown in Fig. 3A. Fitting to a simple binding isotherm yielded average equilibrium dissociation constant estimates of ~10 nM for Nhp6A and ~50 nM for HMGB1(A+B) under our standard conditions. These results are consistent with previous estimates for related proteins^{20;21;55;63;72}.

To measure protein binding cooperativity, longer radiolabeled DNA probes (50 or 200 bp) were used in electrophoretic gel mobility shift assays. The results are shown in Fig. 3B. Nhp6A binding to DNA was cooperative (left panel). The cooperativity parameter was estimated as ~40 as described⁷³. The binding of HMGB1(A+B) also displayed cooperativity (Fig. 3B, center, right), but the data did not permit estimation of a cooperativity parameter due to the absence of discrete intermediate species.

Measurement and modeling of DNA cyclization rate enhancement by HU and HMGB proteins

To determine the effects of HU and HMGB proteins on DNA ring closure we measured the effective concentration of one DNA terminus in the vicinity of the other for a series of six DNA fragments with lengths between 196–206 bp. The DNAs are designed to be intrinsically straight by virtue of direct repeats of 5-bp segments, and the T4 DNA ligase cyclization kinetics of these molecules have previously been analyzed in detail⁶⁶. The *j*-factors were estimated as described⁶⁷. An example of experimental data is shown in Fig. 4A. Results are shown in Fig. 4B and Table 1 for DNA fragments in the absence or presence of 10 nM HU, 40 nM Nhp6A, or 40 nM HMGB1(A+B). The results show the naked DNA to be characterized by *j*-factors oscillating near an average of 7 nM (range from ~1 – 16 nM). Depending on the DNA probe length, HU protein increased the apparent *j*-factor by 10- to 150-fold, Nhp6A protein by 30 to 100-fold, and HMGB1(A+B) protein by 9- to 60-fold. These results dramatically illustrate the ability of these sequence-nonspecific proteins to enhance ring closure (increase the apparent flexibility) of DNA. Fig. 4B also shows data based on simulation. The treatment of protein-bound DNA captures both the magnitude and phasing of the measured *j*-factors. Modeling the ring-closure propensities of the bare DNA used in the experiments, however, requires an elastic model slightly more deformable than that used to fit previous measurements⁶⁶ (see Methods and Supporting Information). The fitted persistence length of 450 Å corresponds to root-mean-square fluctuations of $\pm 5^\circ$ in the roll and tilt angles between adjacent base pairs compared to the $\pm 4.82^\circ$ angular variations associated with the value of 480 Å fitted by other approaches^{58;74} to the cyclization properties of naturally straight DNA. The variation in the twist of base-pair steps corresponds to room-temperature fluctuations of $\pm 5^\circ$ compared to the $\pm 4.1^\circ$ fluctuations introduced in our previous treatment of ideal DNA⁷⁵. Importantly, the published structures of HU- and Nhp6A-bound DNA^{37;45;50} account without change for the observed data. The simulated fit for the structurally uncharacterized HMGB1 complex suggests that the two-box protein induces less deformation in DNA than the artificial two-box protein in the one related NMR complex⁵².

We wished to understand the effect of protein concentration on the enhancement of DNA ring closure. The j -factor measurements were repeated for the 200-bp and 204-bp DNA fragments in the presence of increasing concentrations of Nhp6A, HMGB1(A+B), and HU proteins. The results are shown in Fig. 4C–D and Table 2. Increasing the concentration of the proteins tends to increase the j -factor over the protein concentration range studied. There is a similar increase in the j -factors extracted from Monte-Carlo simulations performed with corresponding increases in the protein binding probabilities (Table S1). The calculated values mirror the greater increase of the j -factor with concentration found experimentally for HU compared to the HMGB proteins, the slightly greater enhancement of the j -factor for HMGB1 vs. Nhp6A, and the greater differences in the concentration effects on HMGB1 compared to Nhp6A found in the cyclization of 200 vs. 204-bp DNA. The computational treatment, however, does not capture the slight decrease in the observed j -factors found for three of the four measurements carried out at very high protein concentrations.

Protein enhancement of apparent DNA flexibility: experiment and simulation

In this work we measure the j -factors of DNA in the absence and presence of the sequence-nonspecific HU architectural protein and HMGB proteins Nhp6A and HMGB1(A+B). We demonstrate for the first time that these architectural proteins dramatically increase this formal measure of apparent DNA deformability. This determination is important since it controls for possible effects of HMGB and HU proteins on end-joining and isolates and quantifies the effect of protein-induced structural deformations and binding levels on overall DNA folding.

Besides measuring the j -factors, we apply a new Monte-Carlo approach^{68;75} to simulate the data and deduce models that account for the effect of protein. The simulations predict that the experimental j -factor maxima, observed at lengths that are not an integral helical repeat, reflect the untwisting and helical dislocation of DNA by the architectural proteins, and that the bending of DNA by protein is the significant factor that enhances the overall magnitude of the j -factors at all of the experimental chain lengths.

The structures of nucleoprotein assemblies generated in the simulations provide new insight into how the random binding of nonspecific proteins on DNA restriction fragments enhances the ease of ring closure. The effects of the different proteins on the computed ring-closure propensities reflect the three-dimensional structures of the different complexes (Fig. 5). The enhancement of chain cyclization mirrors the extent of protein-induced DNA deformation, with the presence of highly bent HU-bound DNA fragments having a much more pronounced effect on the j -factors than the incorporation of the less deformed HMGB1 and Nhp6A complexes. Indeed, HU has a greater effect on DNA cyclization than either the one- or two-box HMGB proteins. It should be noted that the DNA minor groove is recognized differently by HU compared to HMGB domains, and our approach assumes that HU and HMGB proteins are equally sequence-nonspecific in DNA binding. There is some evidence for subtle DNA sequence preferences for HU³⁰ and the HMG-D protein⁷⁶, but it is unclear if such sequence preferences could have played a role in our experiments. The ease of HMGB-induced DNA ring closure increases to the degree found with HU when the less deforming proteins are present in higher concentration in solution or bind with greater likelihood in the computations.

Our model has an implicit assumption that architectural protein binding is transient so that deformed DNA structures rapidly interconvert. This assumption is not absolutely required, but is consistent with biochemical data for undistorted DNA and low concentrations of architectural proteins in the presence of free DNA^{36;60;64}, and allows optimal DNA structures to cyclize in ligation experiments while non-optimal structures continue to sample additional conformations. Interestingly, architectural protein binding may be more

kinetically stable when saturation leads to formation of a protein/DNA filament, or conditions prevent DNA-induced protein exchange^{20;36;38}.

The structural basis for the concentration-dependent increase in the j -factor lies in the uptake of proteins driving successful closure of minicircles (Fig. S3). The enhancement of DNA bending from the added protein-bound fragments presumably contributes to the increased ease of ring closure in the presence of more bulk protein, i.e., the DNA chain ends are more likely to come in contact in the presence of additional protein-induced bends. The conventional fitting of a wormlike DNA model to the same data, attributing the enhancement of cyclization to a decrease in persistence length, forfeits this direct connection to protein/DNA structure. The extracted persistence length depends upon protein concentration and chain length as well as the degree of local DNA deformation⁶⁷. It is accordingly difficult to understand the effects of different proteins on DNA ring closure without a direct computational approach like that taken here.

The precise uptake of protein, like the increase in the j -factor, depends upon both the DNA chain length and the nature of the bound protein-DNA fragments. Whereas the uptake of Nhp6A is highly dependent on chain length, the average number of HMGB1 proteins on the closed minicircles is fairly uniform for a given level of binding (Fig. S3). Interestingly, the number of minicircle-bound proteins at a given bulk protein concentration tends to be slightly greater at the chain lengths with lower j -factors. As previously reported⁶⁸, the number of minicircle-bound HU molecules also increases at higher binding probabilities.

The different protein-induced changes in DNA twist account for the variation in phase of the chain-length dependence of the j -factors. The localized untwisting effected by the binding of HU and Nhp6A underlies the shift in the theoretical chain length (202 bp) predicted and experimentally found to be most likely to cyclize in the presence of these proteins compared to the 200-bp minicircles formed with greatest ease in the presence of HMGB1 or in the absence of protein, i.e., bare DNA. The difference in twist at protein-bound sites also determines the chain-length dependent uptake of Nhp6A vs. HMGB1 on the minicircles. Whereas the binding of the underwound Nhp6A-bound fragments can relieve the torsional stress on DNA chains whose lengths are not an integral multiple of the double-helical repeat, the binding of HMGB1 has no such effect. Like bare DNA, the ends of the HMGB1-bound duplex must twist in order to bring the ends of the duplex in register for ligation. As a result, the overall fold of the Nhp6A-bound minicircles shows less variation with chain length than the HMGB1-bound rings. Minicircles of HU show a similar chain-length-dependent uptake of protein⁶⁸.

The minicircles binding HU stand out from those binding the HMGB proteins in terms of their overall elongated shapes and greater global deformability (Fig. 5 and Table S2), direct consequences of the more pronounced distortions of DNA by HU. The configurational differences are suggestive of potential ways in which other molecules might distinguish the HU- vs. HMGB-bound DNA rings. A protein like H-NS, which is thought to bridge closely spaced duplexes¹⁸, should associate more readily with the long, narrow HU-bound minicircles compared to either the more open HMGB-bound rings or protein-free DNA minicircles of the same length. The HU-bound minicircles also tend to adopt writhed, figure-8-like configurations at chain lengths that differ from an integral helical repeat. The crossovers in such configurations associate preferentially with various ligands⁷⁷ and might affect binding cooperativity. Such ligands may also be able to distinguish the chain-dependent configurational differences in HU-bound DNA minicircles, i.e., open vs. closed forms, predicted by the simulations. The variation in global structure of the HU-bound rings, as measured by the distribution of the writhing number and the variation in the principal axes, is roughly twice that of HMGB-bound rings of the same chain length. (Table S2). The

enhanced deformability may also contribute to the binding of other ligands. Future research can test these ideas.

Materials and Methods

Protein expression and purification

HU protein was the kind gift of Ishita Mukerji. The *S. cerevisiae* Nhp6A coding region was amplified by PCR from yeast genomic DNA and cloned between the *Nco*I and *Bam*HI sites of expression vector pET-15b. A recombinant plasmid encoding rat HMGB1 (boxes A+B) was obtained from P. Sharp. The coding region was subcloned between *Nde*I and *Xho*I sites of expression vector pET-15b after the internal *Nde*I site was mutated by silent site-directed mutagenesis.

Protein binding affinity by fluorescence anisotropy

A 20-bp fluorescein labeled DNA duplex was created by annealing of fluorescein (F)-labeled oligonucleotide LJM-3560 (5'-F-ATGACATGACACGT₂ACGT₂-3') and LJM-3561 (5'-A₂CGTA₂CGTGTCATGTCAT-3'). Fluorescein labeling efficiency was estimated by comparing absorbance measurements at 495nm and 260nm. The 20-bp fluorescein-labeled DNA duplex was purified by electrophoresis through a 12% native polyacrylamide gel. Binding reactions (200 μ L) contained 5 nM fluorescent DNA duplex and the indicated concentrations of HMGB protein dilutions, and binding buffer (4 mM Tris-HCl pH 7.0, 6 mM KCl, 20 μ g/mL BSA, 2 mM MgCl₂).

Cooperativity of DNA binding

A 50-bp duplex DNA was prepared by annealing synthetic oligonucleotides creating recessed 3' termini that were radiolabeled by [α -³²P]- dATP. Binding reactions for electrophoretic gel mobility shift analysis (10 μ L) contained 20 mM Tris-HCl, pH 8, 50 mM NaCl, 1 mM MgCl₂, 1 mM DTT, 0.25 mg/mL bovine serum albumin, 4% (v:v) glycerol, 10 nM radiolabeled duplex DNA probe, and 0–100 nM HMGB protein. Complexes were analyzed by electrophoresis through native polyacrylamide gels.

DNA cyclization kinetics

pUC19-based plasmids contained intrinsically straight ~200 bp sequences⁶⁶. ~400-bp PCR products containing these intrinsically-straight sequences flanked by *Hind*III sites were amplified by PCR and digested overnight with *Hind*III. T4 DNA ligase-catalyzed cyclization reactions were performed at 22°C with 30 nM DNA restriction fragment, the indicated concentrations of HU or HMGB protein, ligation buffer (20 mM Tris-HCl, pH 8.0, 30 mM KCl, 100 μ g/mL BSA, 1.8 mM ATP, 10 mM MgCl₂), and T4 DNA ligase [final concentrations of 50U/mL (DNA alone) or 20U/mL (DNA and HMGB or HU protein)]. After electrophoresis, band quantitation was performed with NIH ImageJ software for Macintosh and *j*-factors estimated as described in supporting information.

Modeling and simulation

A grand-canonical ensemble of HU- or HMGB-bound DNA was generated with Monte-Carlo methods^{68;75}, in order to provide a theoretical estimate of the *j*-factor under the conditions of the experiments. Models of HU-bound DNA were constructed, as described previously⁶⁸, from the six rigid-body 'step' parameters that describe the arrangements of successive base pairs in the known HU-DNA crystal complexes (PDB_IDs 1P51, 1P71, 1P78)³⁷. Base-pair-level models of Nhp6A-bound DNA were similarly constructed from the 'step' parameters of the 20 solution structures of the protein-oligonucleotide complex (PDB_ID 1J5N)⁵⁰. HMGB1-bound DNA was represented by a modified version of the

reported solution structure of DNA bound to covalently joined tandem HMG boxes (PDB_ID 2GZK)⁵². The intervening protein-free DNA is modeled as an ideal elastic rod with torsional and bending moduli consistent with the measured cyclization properties of bare DNA (see Supporting Information for details).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Fig. 1.

Architectural proteins tested here. DNA bending and kinking in protein complexes with (A) bacterial HU (PDB code 1P51)³⁷, (B) yeast Nhp6A (PDB code 1J5N⁵⁰) and (C) mammalian HMGB1 (depicted here based on PDB code 2GZK, an artificial 2-box HMGB protein⁵²). DNA is shown in cyan and protein in red. D. Schematic illustration of proteins studied here. Upper, *S. cerevisiae* Nhp6A; lower, rat HMGB1. HMG boxes and charged domains are indicated. Note that the anionic C-terminus of HMGB1 was not included in the construct studied here.

Fig. 2.

Schematic of protein-enhanced cyclization. Double-stranded DNA (left) is intrinsically stiff. In the presence of architectural proteins (red triangles) an ensemble of interconverting DNA conformations is generated (center), all characterized by shorter end-to-end distances (arrows). These more compact DNA conformations should facilitate cyclization by DNA ligase (right).

Fig. 3. Characterization of HMGB-DNA interactions. A. HMGB protein affinity for DNA monitored by fluorescence anisotropy. Yeast Nhp6A (filled circles and bold line; K_d 10.2 nM). Rat HMGB1(A+B) (open circles and thin line; K_d 47 nM). Representative data from two experiments are shown. B. Cooperativity in protein-DNA binding measured by electrophoretic gel mobility shift assay. The indicated concentrations of HMGB proteins were incubated with 50-bp (left panels) or 200-bp (right panel) radiolabeled DNAs and analyzed by native gel electrophoresis. Complexes with increasing numbers of bound HMGB proteins (n) are labeled.

Fig. 4.

Quantitative effects of HMGB and HU proteins on DNA cyclization. A. Example of cyclization kinetics data for 204-bp DNA probe cyclized for 2, 4, 6, or 8 min in the presence of the indicated concentrations of rat HMGB1(A+B) protein. Labels indicate linear monomer substrate (m), linear dimer (d), linear trimer (t), monomer circle (mc) and circular multimers (cm). B. Experimental j -factors for cyclization in the absence (black squares) and presence of 10 nM HU (yellow circles), 40 nM Nhp6A (red triangles) or 40 nM HMGB1(A+B) (blue diamonds) compared with values simulated under comparable conditions (colored lines). Corresponding j -factors for increasing concentrations of HU, Nhp6A or HMGB1(A+B) are shown for 200-bp (C) and 204-bp DNA probes (D). The data in panel B have also been reviewed elsewhere⁶⁷.

Fig. 5. Simulated protein-bound DNA minicircles. A. Nhp6A. B. HMGB1(A+B), C. HU. Representative cyclized chains are shown for DNA of length 196 bp (top row), 200 bp (middle row), and 206 bp (bottom row). Molecular images, rendered with Chimera⁷⁸. The number of bound protein molecules in the illustrated examples corresponds to the most likely number of bound molecules found in the simulated ensembles. The DNA pathways are generated from Monte-Carlo-sampled base-pair-step parameters using the 3DNA software^{79;80}. Protein-bound DNA is modeled implicitly in terms of the step parameters found in known or modified high-resolution crystal structures (see supporting information for full details).

Table 1

Experimental effects of HMGB proteins on *j*-factors by cyclization kinetics

DNA length (bp)	<i>j</i> -factor ± sd (nM)									
	Free DNA	sd	n	40 nM Nhp6A	sd	n	40 nM rHMGB1	sd	n	10 nM HU
196	4.3	1.0	6	16.5	5.4	4	104.5	9.7	3	212.1
198	13.3	1.8	7	60.2	10.4	4	109.3	31.4	3	167.9
200	15.7	5.4	17	41.2	26.8	8	231.1	24.8	3	179.2
202	4.2	3.0	6	53.7	13.2	3	172.2	36.1	3	294.6
204	1.7	1.2	7	162.7	29.8	3	112.3	11.0	3	283.9
206	4.4	0.4	5	101.1	7.7	3	80.6	34.6	3	173.5

Table 2
HMGB protein concentration-dependent enhancement of experimental *j*-factor

DNA length	<i>j</i> -factor ± sd (nM)									
	[protein]	Nhp6A	sd	n	rHMGB1	sd	n	HU	sd	n
200	0	15.7	5.4	17	15.7	5.4	17	15.7	5.4	17
200	4	14.9	4.8	8	55.5	10.5	3	55.3	7.4	4
200	10	24.1	13.7	8	112.3	43.4	3	179.2	44.6	5
200	40	41.2	26.8	8	231.1	24.8	3	-	-	-
200	60	35.1	25.1	8	181.8	11.5	3	-	-	-
204	0	1.7	1.2	7	1.7	1.2	7	1.7	1.2	7
204	4	3.3	1.2	3	12.7	4.3	3	20.1	2.3	3
204	10	6.6	2.7	3	32.2	7.2	3	283.9	116.9	3
204	40	162.7	29.8	3	112.3	11.0	3	-	-	-
204	60	48.6	7.7	3	241.4	100.1	4	-	-	-