Biophysical Reviews and Letters, Vol. 1, No. 1 (2006) 29–44

World Scientific
www.worldscientific.com

COMPACTION AND SUPERCOILING OF SINGLE, LONG DNA MOLECULES BY HU PROTEIN

BERNHARD SCHNURR

Department of Physics of Complex Systems
Weizmann Institute of Science
Rehovot 76100, Israel

Skirball Institute of Biomolecular Medicine
Program in Molecular Pathogenesis
NYU School of Medicine
New York, NY 10016, USA
schnurr@saturn.med.nyu.edu

CONSTANTINOS VORGIAS

Department of Biochemistry and Molecular Biology
National and Kapodistrian University of Athens
Panepistimiopolis — Zographou
15784 Athens, Greece

JOEL STAVANS

Department of Physics of Complex Systems
Weizmann Institute of Science
Rehovot 76100, Israel
joel.stavans@weizmann.ac.il

Received 2 July 2005

The bacterial cell contains the highly conserved protein HU in abundance. To characterize its architectural role, we studied the elastic behavior of single, supercoilable DNA molecules (tens of kilobases long) in solution with HU from *B. stearothermophilus* (BstHU) by a micromanipulation assay. We point out quantitative yet notable differences to the behavior of HU from *E. coli* (EcoHU) observed by others. Our main contribution here, however, is to characterize the interaction of BstHU with single molecules of DNA in arbitrary states of supercoiling. BstHU clearly distinguishes under- and overwound substrates, breaking the characteristic symmetry in the elastic response of bare DNA. We demonstrate that BstHU shifts the preferred linking number of the complex, consistent with a model in which bound proteins untwist the double helix. The model qualitatively explains various features, such as overall compaction and weaker dependence on supercoiling, by a softening of the DNA to twist and bending. Previously reported reversal of binding effects at protein concentrations above a threshold also extends to supercoiling. All observed effects are highly sensitive to salt concentrations. Their range and magnitude lend HU great versatility in dynamically altering the physical properties and organization of the nucleoid.

Keywords: HU (BstHU); bacterial nucleoid; supercoiling; single DNA elasticity.

1. Introduction

The bacterial chromosome is highly compacted into a distinct nucleoprotein complex called the nucleoid.[1] Its highly dynamic states of compaction and supercoiling are determined by both environmental cues and the physiological state of the cell.[2,3] Several factors play a role in compacting genomic DNA and in controlling nucleoid structure. These include its organization into a number of topologically isolated, supercoiled domains of about 50–100 kilobases each,[4] macromolecular crowding effects,[5] condensation by cations such as spermine, and the interaction with a number of architectural, nucleoid-associated DNA binding proteins.[6] A prominent member of this group is HU,[7,8] a small, basic protein highly conserved from *Mycoplasma* to extremophiles. HU is present in about 30,000 copies in the bacterial cell and binds without sequence specificity throughout the nucleoid.[9,10] Evidence for considerable local bending of DNA by HU is firmly established.[11–14] In addition to its architectural role — the primary focus of this paper — HU has been implicated as a pleiotropic factor in a variety of biochemical processes[15] such as the control of gene expression,[16] DNA replication,[17] and translation.[18]

Early electron microscopy showed crosslinked HU–DNA complexes as beaded structures,[19] suggesting that HU can form nucleosome-like complexes. DNA compaction by HU was later revisited by another imaging technique that showed compact forms of fluorescently-stained DNA molecules upon addition of HU.[20] Since then, more sophisticated techniques have explored this interaction by micromechanical and other assays[21–23] and conclusively demonstrated that HU is able to compact DNA at large scales. Single DNA techniques are a natural approach to studying the bacterial nucleoid which consists intrinsically of a single DNA molecule.

Micromechanical assays add new dimensions to the characterization of protein binding to DNA and are able, for example, to mimic the biologically relevant forces generated by DNA processing enzymes. Our technique of choice, magnetic tweezers,[24] allows independent stretching and twisting of individual DNA molecules tethering a magnetic bead to a surface and sensitively detects variations in the elasticity of DNA tethers with minimal interference or artifacts, while allowing changes in solution conditions, applied pulling force, and the degree of supercoiling at will. It has been used successfully to probe the elastic behavior of long DNA molecules in solution with HU from *E. coli*.[22,23] Although possible, these studies have so far neglected to characterize HU binding with respect to supercoiling. Lia *et al.* have studied the effect of supercoiling on HU-mediated looping[25] but without separately characterizing HU. Our present study addresses this aspect, using magnetic tweezers, and extends investigations of the interaction of HU with supercoiled DNA in bulk[26,27] to the single DNA level.

The literature on HU has primarily focused on HU from *E. coli* (EcoHU)[8] but increasingly also addresses forms of the protein from other sources, especially from thermophiles such as *B. stearothermophilus* (BstHU), the subject of this study and the source of the first crystallographic structure of any HU protein.[28]

Work characterizing HU has often reached seemingly contradictory conclusions, at least in part because the HU variants are rather different proteins. EcoHU and BstHU, for example, share only about 60% sequence identity.[29] Together with a strong salt dependence, the range and sensitivity of HU binding effects, e.g. compaction/decompaction depending on protein concentration (bimodality), surely contribute to the incongruence of reported results concerning cooperativity, binding strength, and binding equilibrium.

In Sec. 3 we present the basic elastic behavior of BstHU and make the comparison to similar studies with EcoHU using the same technique.[22,23] BstHU also compacts DNA, indeed much more strongly than EcoHU. As for EcoHU, compaction is increasingly relieved above a threshold protein concentration. Unlike for EcoHU, however, we find no stiffening of the DNA–protein complex *beyond* bare DNA levels in the presence of BstHU under the conditions tested.

Our major results, aspects of the interaction of BstHU with supercoiled DNA, make up Sec. 4. The central observation is that binding of BstHU breaks the established symmetry[24] in the elastic response of individual bare DNA molecules to over- and underwinding (under moderate stretching forces). The weaker response to supercoiling is attributed to a notable softening to both bending and twist deformations in the presence of BstHU. Our measurements also demonstrate a force-dependent shift in the preferred twist of the DNA–protein complex, as predicted by a model for proteins that unwind DNA upon binding.[30] Bimodality extends to our supercoiling assay as well. Based on this mechanical single DNA assay, we understand both asymmetry and shift as clear evidence for local untwisting of the double helix by BstHU.

2. Materials and Methods

2.1. *HU protein and DNA substrates*

The gene for HU from *B. stearothermophilus* (BstHU) was overexpressed in *E. coli*[31] and highly purified, as analyzed by SDS–PAGE. *E. coli* HU (EcoHU), used in an informal comparison between the two proteins, was a gift from J. Rouvière-Yaniv and S. Adhya. Supercoilable (torsionally constrained) DNA molecules were prepared following the approach pioneered by Strick *et al.*[24] Our substrate was based on the pCEP4 vector (Invitrogen), a gift from A. Oppenheim. The plasmid was first digested with BamHI/HindIII and the linearized product ($\sim 10.4\,$kb) then selectively ligated to short DNA fragments ($\sim 0.7\,$kb) multiply labeled with either digoxigenin or biotin. The end fragments were produced by PCR with a nucleotide mixture including a fraction of covalently labeled dUTPs (Digoxigenin–11–dUTP and Biotin–16–dUTP, Roche Molecular Biochemicals) and digested to match the left and right ends of the linearized plasmid, respectively. Experiments that did not require supercoiling were duplicated with longer tethers made from λ DNA ($\sim 48.5\,$kb, Roche Molecular Biochemicals) as described previously.[32] Finally,

poly[dI–dC] (Polydeoxyinosinic-deoxycytidylic acid, Sigma-Aldrich) at 0.02 mg/ml served as a binding competitor substrate to probe HU unbinding.

2.2. *DNA tethers and flow cell preparation*

DNA tethers for micromanipulation were prepared by incubating 2.8 μm tosyl-activated paramagnetic beads (Dynabeads M-280, Dynal) with anti-digoxigenin (polyclonal antibody, Roche Molecular Biochemicals). These beads were conjugated to the digoxigenin ends of the DNA substrates, leaving their biotin ends available to specifically tether the bead to the streptavidin-coated surface of the sample cell. Forming multiple attachment points between bead (or surface) and its matching DNA end ensures that bead rotations transmit torsional strain to the tether rather than relax it by swiveling.

Flow cells were prepared as described previously:[32] glass capillaries of square cross section (Microcells, VitroCom Inc.) were coated by incubation with BSA–biotin followed by streptavidin. DNA–bead constructs were then flowed in and left to bind. All measurements were performed at pH 7.4 in the same buffer: 10 mM Tris-HCl, 0.1 mM EDTA, 5% DMSO, 0.2 mg/ml alpha casein, and either 200 or 100 mM KCl, as indicated. Different protein concentrations in the flow cell were achieved by complete buffer exchange, flowing in a volume of $\sim 150\,\mu$l (about ten times the capillary volume). All experiments were conducted at room temperature.

2.3. *Magnetic tweezers and elasticity measurements*

Flow cells containing DNA-tethered magnetic beads were observed under bright-field illumination using a custom-built inverted microscope,[32] with the addition of a set of external magnets whose position and rotation was computer-controlled. Elasticity measurements were carried out following established techniques and protocols.[24,32] Magnetic beads transduce force and torque from external magnets to the DNA tether. The magnetic field gradient in the sample is modulated by changing the distance of the external magnets from the flow cell and results in forces (~ 0.03 to 30 pN) applied to the free (bead) end of each DNA molecule. The direction of the field imposes an orientation on the magnetic beads that faithfully follow magnet rotations, thus supercoiling the DNA to arbitrary degrees while tethered in solution. Stretching forces were calibrated for each tether using the bead's Brownian motion. DNA extensions (projected end-to-end distances along the direction of force) were found by correlating bead images acquired with a CCD camera to a library of images of the same bead under high force (> 10 pN). Library images were acquired by moving the microscope objective (Plan-Apochromat, 63×1.40 NA oil immersion, Zeiss) with a closed-loop piezoelectric mount (MIPOS 3 SG, Piezosystem Jena). Calibrations were made for every tether and compared to the well-known force-extension characteristics of single DNA molecules. Our data are the result of two types of assays: the measured quantity is always the DNA extension while either the applied force or the degree of

supercoiling (at fixed force) are varied. Since only the supercoiling assay requires twist-constrained tethers, the force-extension characteristics were also measured with unconstrained tethers. We found no significant differences either due to tether length or bead attachment.

2.4. *Data acquisition and analysis*

Image acquisition and computer-controlled stepper motors were integrated using LabVIEW (National Instruments). Series of extension measurements in the direction of increasing and decreasing forces were compared to exclude instrumental drift and hysteresis. Data shown are averages of at least four such series. We estimate the error in our measurements at about 1% of the total tether length. Data shown in a figure under the same buffer conditions were measured with the same tether.

Values for the effective bending and twist persistence lengths A and C were determined from the extension data in Fig. 2A using fits to Eq. (1) around the maxima. For each data set, the position of the maximum determined the shift n_0 and bending persistence length A independently. With A determined, C could be calculated from the curvature of the parabolic fit. Bending persistence lengths A were independently confirmed from force-extension data of the same tether using the linear relation between $f^{-1/2}$ and the extension z in the high-force limit.[33]

3. Basic Elasticity and Compaction by BstHU

3.1. *Strong compaction of single DNA molecules at large scales*

We measured the elastic characteristics of individual DNA tethers at various concentrations of BstHU in solution and for two salt concentrations (100 and 200 mM KCl) flanking physiological values. To emphasize deviations from the well-known bare DNA behavior[24] we plot extensions relative to those of bare DNA (at the same forces, see Fig. 1). In qualitative agreement with observations of single HU–DNA complexes in solution by fluorescence microscopy[20] and single molecule elasticity measurements of EcoHU–DNA complexes by others,[22,23] we find that BstHU also induces DNA compaction. In fact, we compared EcoHU and BstHU under identical conditions and found that compaction by BstHU far exceeds that of EcoHU: to achieve comparable compaction in the same buffer required a hundredfold higher concentration of EcoHU than BstHU (data not shown, as our results confirm those from previous work[22,23]). This increased effectiveness is clearly biologically relevant as BstHU is charged with maintaining the nucleoid of a thermophilic bacterium that lives at much higher ambient temperatures than *E. coli*.

The basic result is that increasing concentrations of BstHU (below a threshold, discussed in the next section) lead to greater compaction. Under all the conditions we tested, DNA with protein was more compact than bare DNA, as reflected in relative extensions less than one. At the highest forces (10 pN and above), where the

protein is apparently no longer able to bend its substrate effectively, all measured extensions converge towards the tether's full contour length.

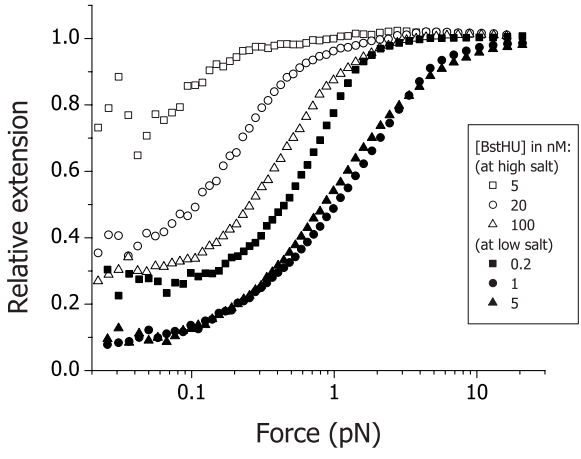
Notably, if not altogether surprisingly, due to the largely electrostatic nature of the interaction, the degree of compaction depends strongly on monovalent salt levels. For example, the compaction induced by 0.2 nM BstHU at low salt (100 mM KCl) is comparable to that induced by roughly 500 times that concentration at high salt (100 nM HU with 200 mM KCl). By contrast, Skoko *et al.*[22] report that the salt-dependence for EcoHU is rather weak.

HU is a highly charged (basic) protein. Yet the relative effectiveness of BstHU is not due to straightforward electrostatics. In fact, the aminoacid sequence of BstHU contains fewer net positive charges (8, for the homodimer) than that of EcoHU (15, for the heterodimer). We presume it is the spatial distribution of these charges that results in such distinct behavior. It is also possible that, beyond general screening of the electrostatic interaction between HU and DNA, high salt concentrations specifically disrupt salt bridges, as in the case of IHF, a closely related nucleoid protein.[34] Whatever the mechanism for the superior stability and binding of BstHU, we suspect it at least partly explains why the crystal structure of BstHU[28] was the first among the HU proteins to be solved.

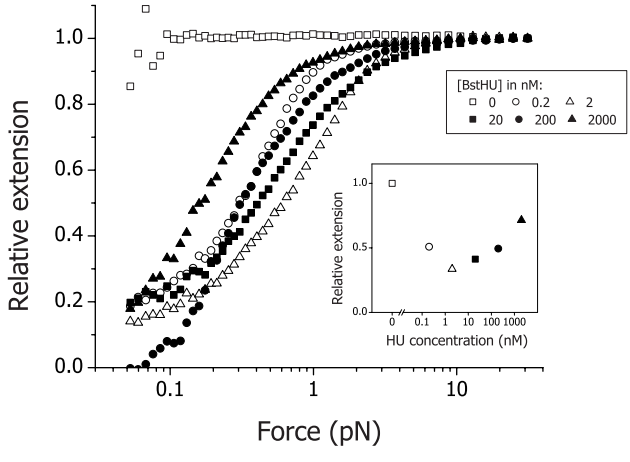
3.2. *Protein concentrations above a threshold counteract DNA compaction*

Single DNA micromanipulation experiments on EcoHU by others[22,23] have shown both compaction and effective stiffening beyond bare DNA levels at very low salt concentrations. Increasing BstHU concentration above a threshold also partially relieves DNA compaction in our experiments (Fig. 1B). In stark contrast to the cited work on EcoHU, however, even the addition of up to 2 μM BstHU in low salt buffer (100 mM KCl) never extended our tethers beyond bare DNA levels, or came even close. Under low salt conditions (100 mM KCl) this threshold is around a few nanomolar, and around a few hundred nanomolar at higher salt concentrations (200 mM). We recapitulate both compaction and decompaction in the figure inset, where DNA extensions at a moderate force (0.3 pN) are shown as a function of protein concentration. Given that EcoHU and BstHU share only about 60% sequence identity, we hypothesize that the differences we describe here reflect genuine and noteworthy distinctions between the two versions of HU rather than merely variations in the experimental conditions.

We point out that the elastic behavior below and above the critical concentration does not bear the same functional form. This suggests that complexes formed at high protein concentrations differ structurally from those at lower concentrations, as has been argued in Refs. 21 and 22. Cooperativity may even lead to binding in polymerized structures along the DNA rather than as individual dimers, as evidenced by atomic force microscopy.[23,35] To reflect the competing effects on DNA elasticity, we refer to binding in these two modes as "bimodal".[22] Other examples



(A)



(B)

Fig. 1. Concentration- and salt-dependence of DNA compaction by BstHU protein. (A) Comparison of the extensions (relative to that of bare DNA) of single tethers in solution with protein as a function of the force applied to the ends of a λ DNA molecule, for two salt concentrations. For high salt conditions (200 mM KCl) we use open symbols for protein concentrations of 5 (squares), 20 (circles), and 100 (triangles) nM BstHU. Measurements at low salt (100 mM KCl) are represented by full symbols for BstHU concentrations of 0.2 (squares), 1 (circles), and 5 (triangles) nM. Experimental noise increases notably towards the lowest forces due to larger bead fluctuations. We note a threshold concentration for compaction at low salt around a few nM. (B) Force-extension characteristics of a shorter tether ($\sim$10 kb) that demonstrate compaction (open symbols) and decompaction (full symbols). The measurement covers four orders of magnitude in protein concentration (at 100 mM KCl): 0.2 (open circles), 2 (open triangles), 20 (full squares), 200 (full squares), 2000 (full triangles) nM BstHU. Following the addition of poly[dI–dC] as a competitor substrate at 0.02 mg/ml (open squares) we recover bare DNA elasticity. Inset: extensions at 0.3 pN (taken from the main figure, matching symbols) are replotted as a function of protein concentration to illustrate the initial compaction followed by decompaction beyond a threshold concentration of a few nM.

of bimodal (or non-monotonic) behavior have been reported in FRET (Fluorescence Resonance Energy Transfer) experiments of HU complexes with 55 bp DNA oligomers[21] as well as in the elastic response to supercoiling described in Sec. 4.4 of this paper.

3.3. *BstHU does not continually bind and unbind DNA in thermodynamic equilibrium*

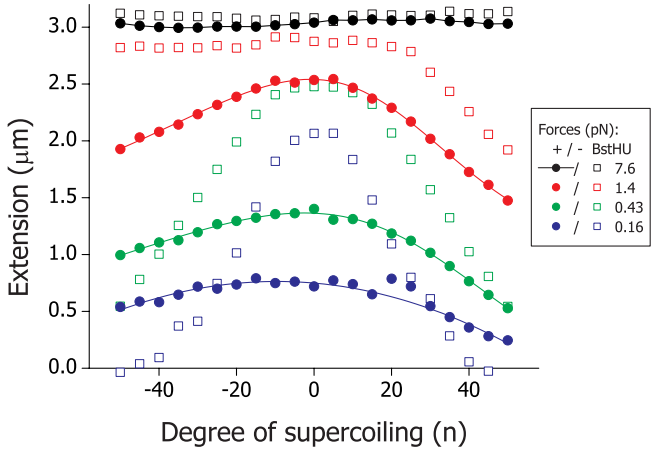
Skoko *et al.*[22] have suggested that, in one mode of binding, rather than continually binding and unbinding in thermodynamic equilibrium with the protein in solution, EcoHU unbinds almost exclusively via collisions with free DNA in solution. In addition, they report finding conditions (at low protein concentrations during initial binding) where EcoHU does come on and off the DNA in equilibrium.

In our experiments, even under more stringent conditions (higher salt concentrations) than those used with EcoHU by others, BstHU remains tightly bound to DNA on experimental time scales of hours. Throughout our experiments we found that dilution of BstHU with buffer (even with vigorous flushing under high force or twist) did not remove bound protein from a tethered DNA molecule, as was evident from our inability to recover bare DNA elastic behavior. Thus, convergence towards the DNA contour length at high stretching forces is likely not due to the unbinding of protein but to the deformation of HU-induced bends. The introduction of competitor DNA in solution, however, almost immediately returned the tether's elasticity to that of bare DNA (see Fig. 1B). By contrast, dilution was sufficient to recover bare DNA elasticity in experiments with the related nucleoid protein IHF (R. Amit, unpublished results). This difference may be due to cooperativity exhibited by HU but not by IHF. The absence of equilibrium binding and unbinding in the case of BstHU argues strongly for cooperative interactions, a hypothesis supported by FRET experiments on the same protein.[21]

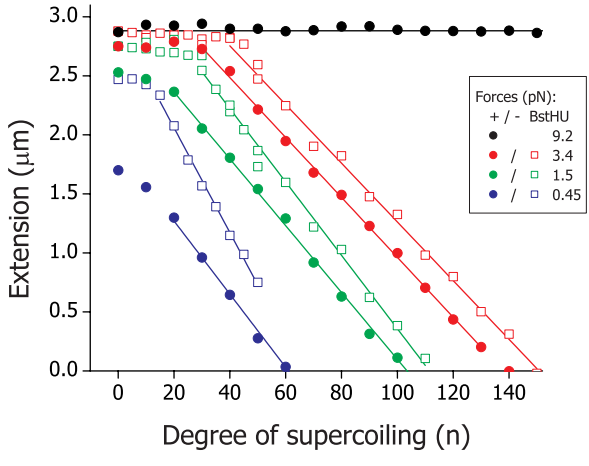
4. BstHU and Supercoiled DNA

4.1. *Compaction by BstHU depends on whether DNA is overwound or underwound*

Having made the comparison between the basic elastic behavior of DNA in the presence of BstHU or EcoHU (as reported by others[22,23]) in Sec. 3, we now extend the characterization of BstHU to its interaction with supercoiled DNA, its natural substrate in the bacterial cell. To test the supercoiling dependence of BstHU we attached each tether end at multiple points to yield torsionally constrained DNA. Arbitrary degrees of supercoiling can then be produced in the same molecule by rotating the tethering bead with external magnets. In this assay, we measure extensions for integer numbers of complete turns n in both directions, with positive n referring to overwinding and negative n to underwinding of the double helix.



(A)



(B)

Fig. 2. Effects of BstHU on the elastic behavior of supercoiled substrates. Panel (A) compares extensions measured for DNA tethers without protein (open squares) and with BstHU (full circles) as a function of the degree of supercoiling for a set of four forces: 0.16 (blue), 0.43 (green), 1.4 (red), and 7.6 (black) pN. The lines through the data with protein are merely to guide the eye. Protein and KCl concentrations in these measurements were 100 nM and 200 mM respectively. Panel (B) continues similar extension measurements without protein (open squares) and with BstHU at 20 nM (full circles) to much higher degrees of overwinding (up to almost 15%). The forces here are 0.45 (blue), 1.5 (green), and 3.4 (red) pN. For the experiments with protein we show an additional data set taken at 9.2 pN (black) to demonstrate that there is no compaction, at sufficiently high forces, even for these large degrees of overwinding. Lines represent linear fits over the range shown. All data were obtained at high salt (200 mM KCl) using a $\sim$10 kb tether, where 10 turns correspond to a 1% change in the degree of supercoiling.

Figure 2A shows extensions as a function of supercoiling for a number of forces, both with and without protein in solution.

We found that the supercoiling-induced reduction in extension in the presence of BstHU at moderate forces (0.16 and 0.43 pN) clearly depends on whether the DNA substrate is overwound or underwound. That is, the data (full circles in Fig. 2A) are *asymmetric* with respect to $n = 0$. The data appear tilted towards overwinding. The presence of protein thus breaks the symmetry that characterizes the well-known hat-shaped response of bare DNA at low forces[24] (open squares in Fig. 2A). Underwound DNA *without* protein is the exception as it fails to compact DNA at intermediate forces (e.g. at 1.4 pN, red open squares in Fig. 2A). Unrelated to any protein effect, this is understood to reflect bubble formation in the underwound double helix. We elaborate on this issue and on the implications of HU's binding preference below. Here, we suggest that the broken symmetry in the presence of BstHU directly reflects the protein's preferential affinity for negatively supercoiled DNA. Unlike previous work in bulk that has proposed such a binding preference,[19,26,27] our assay provides mechanical control over and a direct measurement of a single DNA molecule in solution. This allows us to study the behavior for any degree of supercoiling not only with the same DNA sequence but on the very same molecule.

4.2. *Bound BstHU softens DNA to twist and bending*

A distinctive feature of the elastic response of BstHU–DNA complexes seen in Fig. 2A, beyond the overall compaction at $n = 0$, is that the decrease in extension with n is shallower in the presence of BstHU than for bare DNA. To confirm this, we extended our measurements to higher degrees of overwinding (positive n) for a number of forces. Figure 2B indeed demonstrates that the shallower slopes for DNA with protein are maintained to very high degrees of supercoiling, up to 15% here. In addition, the figure illustrates the expected linearity of the "wings" very clearly. Linear reduction in extension with supercoiling is a signature of plectoneme growth.[24,36] We argue below that the weaker response to supercoiling of DNA–protein complexes (compared to bare DNA) is due to an effective softening of the DNA within the complex.

Two important material properties that govern the elastic response are the twist and bending moduli. We estimate their effective values for the BstHU–DNA complex from our data using the result of a theory of proteins that bend and twist DNA upon binding.[37] Like the work it is based on,[38,39] this model, known as the "twisted wormlike chain" or the "torsional directed walk", adds torsional stiffness to the conventional description of polymer conformations. The theory applies only to modest degrees of supercoiling (in the absence of plectonemes) and to low protein occupations, where protein–protein interactions along the DNA are negligible. In this formulation, both persistence lengths are left unconstrained to account for the effects of binding proteins. Ignoring twist-bend and twist-stretch couplings, the

average extension z of a supercoiled complex is given by:

$$\frac{z}{L} = 1 - \sqrt{\frac{k_B T}{4Af}} - \frac{(2\pi C)^2}{2L^2}\left(\frac{k_B T}{4Af}\right)^{3/2}(n - n_0)^2 \qquad (1)$$

where L is the contour length of the DNA substrate, f the force applied at its ends, A and C the effective bending and twist persistence lengths, measures related to the bending and twist moduli via the thermal energy $k_B T$. The parameter n_0 allows for an overall shift or offset, a generalization introduced in anticipation of the effects of protein binding. Fitting the central regions of the two low-force data sets from Fig. 2A to this function (as described in "Materials and Methods", Section 2) yields $A = 10 \pm 1$ nm and $C = 7 \pm 1$ nm for 0.16 pN, and $A = 7 \pm 1$ nm and $C = 15 \pm 1$ nm for 0.43 pN (at 100 nM BstHU and 200 mM KCl). These values are considerably lower than those for bare DNA at the same forces ($A = 40 \pm 3$ nm and $C = 87 \pm 4$ nm), values that agree with published data.[39, 40] We independently confirmed the bending persistence lengths A from force-extension characteristics of the same tethers and found 11±3 nm with protein and 43±4 nm without protein. A systematic force dependence of the persistence length fits indicates that a complete description requires a more elaborate model. The twist persistence length C shows a residual increase with force and eventually approaches bare DNA values at the highest forces. A possible explanation for this is that C does not bear any intrinsic force dependence, for example due to distortions of the DNA–protein complexes at high tensions leading to nonlinear elasticity. If the softening to twist is due to melting of the double helix where HU bends, then higher forces would indeed increasingly suppress this effect as bends get straightened out. Despite these limitations, we can conclude that locally bound BstHU protein changes the effective mechanical properties of DNA considerably, softening the double helix to both bending but even more strongly to twist deformations.

To complete the argument for the weaker dependence of the elastic response on supercoiling in the presence of BstHU, we consider the functional dependence of Eq. (1) on A and C. The twist contribution dominates as C enters with a power of 2, greater than the 3/2 for A. Together with the dominant reduction in C (compared to A) this leads to a smaller prefactor of the quadratic term in Eq. (1) and thus to the weaker dependence of extension on supercoiling (Fig. 2A, full circles). We qualitatively expect the softening of the double helix (especially to bending) to lead to slower rates of plectoneme formation with n, as the size of plectonemic loops decreases with the bending modulus.[24]

Returning to the remaining data in Fig. 2A, BstHU *does* compact underwound DNA at intermediate forces (1.4 pN), in stark contrast to the case of bare DNA, where the extension remains constant under those conditions due to the nucleation and growth of denaturation bubbles.[24] We infer that BstHU at least partially inhibits the formation of denaturation bubbles and effectively stabilizes the double helix, a potentially useful feature particularly for a thermophile. The failure of underwound DNA to be compacted at intermediate forces is notably the only

exception to the otherwise symmetric elastic behavior of bare DNA with respect to under- and overwinding. That asymmetry stems from the chiral nature of the DNA molecule itself. What we describe here, however, is due to the binding of BstHU and reflects its unwinding of the DNA. We cannot rule out that protein binding to single-stranded regions (within bubbles) and subsequent bending contributes to the reduction in extension. Finally, at higher forces (several pN), the response of DNA with and without protein shows no dependence on supercoiling at all, only a slight overall compaction with protein, as in Fig. 1.

4.3. *Shifts in extension peaks demonstrate that BstHU untwists the DNA double helix*

Yan and Marko have calculated the effects of DNA-distorting proteins on the extension of DNA molecules subject to supercoiling.[30] Their numerical studies predict that DNA-untwisting proteins shift the peak in extension from negative n at small forces to positive n at larger forces. For bare DNA, by comparison, all extension maxima coincide at $n = 0$, independent of stretching force.

Indeed, BstHU–DNA complexes exhibit the predicted shift in the preferred twist, as shown in Fig. 3 for two different protein concentrations. Again, the size of the effect is strongly salt-dependent: contrast the considerable shifts for the data in Fig. 3 (at low salt) with the very minor shift in Fig. 2A (at high salt). At the lower protein concentration in Fig. 3 (2 nM BstHU, open squares) the extension

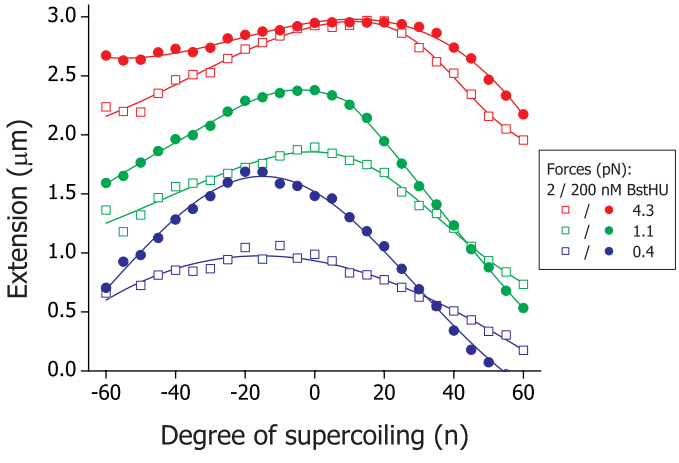


Fig. 3. Peaks in the extension of supercoiled DNA shift with force. DNA extensions as a function of supercoiling are shown for 2 (open squares) and 200 (full circles) nM BstHU and stretching forces of 0.4 (blue), 1.1 (green), and 4.3 (red) pN. The increasing shift to the right with force confirm theoretical and numerical predictions for proteins that locally untwist the double helix upon binding. Lines through the data are merely to guide the eye. Measurements were carried out at low salt (100 mM KCl) using a $\sim$10 kb tether, where 10 turns correspond to a 1% change in the degree of supercoiling.

peak shifts from $n_0 = -12 \pm 2$ to 7 ± 1 at 0.4 and 4.3 pN respectively, a significant change in linking number of almost 2%. For reference, genomic DNA in bacteria is typically underwound by about 5%, about half of which is thought to be absorbed in DNA binding proteins such as HU.[3] While HU is neither the only protein in bacteria with this role nor available in sufficient numbers to densely cover the entire genome, unlike in many *in vitro* assays, the magnitude of this shift indicates that HU has considerable potential to modulate supercoiling within the nucleoid.

Physically, this shift arises from force-dependent changes in the relative importance of chiral and bending modes in reducing the extension. Below the onset of plectoneme formation (i.e. for weak supercoiling), small changes in linking number impart torsional stress to the DNA tether. The resulting deformation is partitioned into twist and writhe, depending on the applied force.[41] For small forces, linking number changes go predominantly into writhe, while high forces shift this balance towards twist. Of the two, only writhe reduces DNA extension, even below the onset of plectoneme formation, where the tether takes on a stretched solenoidal shape. Local untwisting by HU relieves some of this torsional stress, but only in underwound substrates. This relaxation results in maximal extensions for underwound DNA (negative n) at small forces. At large forces, on the other hand, writhe is negligible compared to twist and the dominant reduction in extension is due to bending by HU. As HU binds preferentially to underwound substrate, based on gel shift assays,[27] compaction is weaker for overwound DNA (positive n) and extension peaks shift to larger n (Fig. 3).

Based on the available crystal structures[7,12,28] and evidence from bulk assays with circular DNA[19,26] it has been argued that HU locally untwists DNA. In our experiments, we demonstrate this effect at the single molecule level, in solution, and with control over the state of supercoiling in the DNA substrate. This makes the assay reversible and allows us to probe the effect not only with the same nucleotide sequence but with the very same molecule of DNA. In addition, our results provide direct experimental verification of a critical prediction from a numerical model describing proteins that locally unwind DNA upon binding.[30]

4.4. *Bimodal effects extend to supercoiled DNA*

Having seen in the force-extension data that HU-induced compaction of DNA is partially relieved above a threshold concentration (Fig. 1B), we looked for similar non-monotonic behavior in the supercoiling assay. Figure 3 contrasts supercoiling at concentrations below and above the threshold (2 and 200 nM BstHU, at 100 mM KCl). At least for the lower forces, we clearly see decompaction above threshold concentrations of HU for small degrees of supercoiling. That is, near the center of the hat curves in Fig. 3, the sets of full circles reflect a more extended tether. However, the change in extension for increasing under- or overwinding is markedly stronger at the higher HU concentration. This is the opposite of our previous observation at higher salt concentration: the addition of BstHU *below* the threshold results

in *shallower* hat curves (Fig. 2A) and a *weaker* dependence on increased overwinding (Fig. 2B). We conclude that non-monotonic effects leave their signature in the elastic behavior of supercoiled DNA as well. Not only is the compaction with protein concentration reversed above the threshold, but the hat-shaped response characteristic of supercoiling at low forces grows shallower below a threshold protein concentration and steeper above it.

5. Conclusions

HU is a major nucleoid-associated protein whose structural effects on DNA cover a wide range. Our work addresses the effects of the BstHU variant (HU from *B. stearothermophilus*) on DNA elasticity at large scales (tens of kilobases). Reversibility in all aspects, including solution conditions, and independent mechanical control over pulling force and supercoiling in a long DNA molecule in solution, held by its ends, make the technique we employ here particularly well-suited to the characterization of DNA–protein interactions.

Our studies show that the thermophile BstHU compacts DNA very strongly. In fact, the compaction (at room temperature) is considerably stronger than that effected by the mesophile EcoHU, observed previously in studies without control over supercoiling.[22,23] Decompaction for protein concentrations beyond a threshold, however, does not extend to a stiffening beyond bare DNA levels, as seen with EcoHU.[22] We also note the virtual inability to unbind the protein from its DNA substrate except through direct contact with free DNA in solution. This property should be relevant to the mode of distribution and transport of HU within the nucleoid.[42] Furthermore, it illustrates that DNA–protein interactions cannot necessarily be treated in the context of thermodynamic equilibrium.

Section 4 then introduces controlled supercoiling (to arbitrary degrees) to the characterization of HU binding effects on DNA. Our measurements show that the state of supercoiling of a DNA molecule is intimately linked to its complex formation with BstHU. As a result, the characteristic symmetry in the elastic response of bare DNA to over- and underwinding is broken in the presence of this protein. Both assays we describe, monitoring DNA compaction as a function of either supercoiling or force applied at the ends of the tether, display non-monotonic (bimodal) behavior with protein concentration. Binding effects at low concentrations are increasingly reversed beyond a characteristic threshold, likely reflecting a different binding mode with more prominent cooperativity.

Quantitative comparison to a twisted wormlike chain model[37] reveals that one important effect of BstHU is the softening of the double helix to both twist and bending deformations. This softening likely plays a role in the HU-assisted loop formation associated with the Gal repressosome.[25,43] Finally, a stretching-force dependent shift in the elastic response of supercoiled complexes shows unequivocally that their preferred twist is different from that of bare DNA. Shifted extension peaks indeed confirm a prediction from numerical work on the elasticity of DNA–protein

complexes[30] and establish, in a reversible experiment at the single molecule level, that bound BstHU untwists the double helix.

Bacteria, whose nucleoid is dynamically maintained in a supercoiled state, must benefit from the remarkable range in the modulation of nucleoid organization, both in terms of overall compaction and local supercoiling. It is known that transcription induces segregated domains of positive and negative supercoiling.[44] The sensitivity of BstHU to and its effect on the state of supercoiling of the DNA substrate suggests that this protein plays an important role in the control of gene expression as well as in other important regulatory and organizational mechanisms in cells. While it is problematic to extrapolate the conclusions from *in vitro* experiments to the complex and crowded environment of the cell's interior, the wide range of effects we observe makes HU invaluable to the bacterial cell for the dynamical modulation of nucleoid structure, including in response to environmental cues.

Acknowledgments

We are grateful to support, and T. Strick for advice regarding the preparation of supercoilable substrates. Our thanks also go to J. Marko, D. Bensimon and V. Croquette for valuable discussions. This study was supported by The Israel Science Foundation (grant no. 320/04).

References

1. N. J. Trun and J. F. Marko, *ASM News* **64**, 276 (1998).
2. M. Thanbichler, P. H. Viollier and L. Shapiro, *Curr. Opin. Genet. Dev.* **15**, 153 (2005).
3. D. E. Pettijohn, The Nucleoid, in *Escherichia coli and Salmonella: Cellular and Molecular Biology*, eds. F. C. Neidhardt, R. Curtiss III, J. L. Ingraham, E. C. C. Lin, K. B. Low, B. Magasanik, W. S. Reznikoff, M. Riley, M. Schaechter and H. E. Umbarger, Vol. 1, 2nd edn. (American Society for Microbiology, Washington, D. C., 1996), p. 158.
4. R. R. Sinden and D. E. Pettijohn, *Proc. Natl. Acad. Sci. USA* **78**, 224 (1981).
5. T. Odijk, *Biophys. Chem.* **73**, 23 (1998).
6. T. A. Azam and A. Ishihama, *J. Biol. Chem.* **274**, 33105 (1999).
7. K. K. Swinger and P. A. Rice, *Curr. Opin. Struct. Biol.* **14**, 28 (2004).
8. J. Rouvière-Yaniv and F. Gros, *Proc. Natl. Acad. Sci. USA* **72**, 3428 (1975).
9. T. A. Azam, S. Hiraga and A. Ishihama, *Genes Cells* **5**, 613 (2000).
10. M. Wery, C. L. Woldringh and J. Rouvière-Yaniv, *Biochimie* **83**, 193 (2001).
11. D. Serban, J. M. Benevides and G. J. Thomas, *Biochemistry* **42**, 7390 (2003).
12. K. K. Swinger, K. M. Lemberg, Y. Zhang and P. A. Rice, *EMBO J.* **22**, 3749 (2003).
13. K. Wojtuszewski, M. E. Hawkins, J. L. Cole and I. Mukerji, *Biochemistry* **40**, 2588 (2001).
14. K. Wojtuszewski and I. Mukerji, *Biochemistry* **42**, 3096 (2003).
15. B. D. Lavoie, G. S. Shaw, A. Millner and G. Chaconas, *Cell* **85**, 761 (1996).
16. D. E. A. Lewis, M. Geanacopoulos and S. Adhya, *Mol. Microbiol.* **31**, 451 (1999).
17. A. Bahloul, F. Boubrik and J. Rouvière-Yaniv, *Biochimie* **83**, 219 (2001).
18. A. Balandina, L. Claret, R. Hengge-Aronis and J. Rouvière-Yaniv, *Mol. Microbiol.* **39**, 1069 (2001).
19. J. Rouvière-Yaniv, M. Yaniv and J. E. Germond, *Cell* **17**, 265 (1979).

20. T. Endo, N. Sasaki, I. Tanaka and M. Nakata, *Biochem. Biophys. Res. Commun.* **290**, 546 (2002).
21. D. Sagi, N. Friedman, C. Vorgias, A. B. Oppenheim and J. Stavans, *J. Mol. Biol.* **341**, 419 (2004).
22. D. Skoko, B. Wong, R. C. Johnson and J. F. Marko, *Biochemistry* **43**, 13867 (2004).
23. J. van Noort, S. Verbrugge, N. Goosen, C. Dekker and R. T. Dame, *Proc. Natl. Acad. Sci. USA* **101**, 6969 (2004).
24. T. R. Strick, J. F. Allemand, D. Bensimon, A. Bensimon and V. Croquette, *Science* **271**, 1835 (1996).
25. G. Lia, D. Bensimon, V. Croquette, J.-F. Allemand, D. Dunlap, D. A. E. Lewis, S. Adhya and L. Finzi, *Proc. Natl. Acad. Sci. USA* **100**, 11373 (2003).
26. S. S. Broyles and D. E. Pettijohn, *J. Mol. Biol.* **187**, 47 (1986).
27. H. Shindo, A. Furubayashi, M. Shimizu, M. Miyake and F. Imamoto, *Nucleic Acids Res.* **20**, 1553 (1992).
28. I. Tanaka, K. Appelt, J. Dijk, S. W. White and K. S. Wilson, *Nature* **310**, 376 (1984).
29. M. Kimura and K. S. Wilson, *J. Biol. Chem.* **258**, 4007 (1983).
30. J. Yan and J. F. Marko, *Phys. Rev. E* **68**, 011905 (2003).
31. P. M. Padas, K. S. Wilson and C. E. Vorgias, *Gene* **117**, 39 (1992).
32. B. M. J. Ali, R. Amit, I. Braslavsky, A. B. Oppenheim, O. Gileadi and J. Stavans, *Proc. Natl. Acad. Sci. USA* **98**, 10658 (2001).
33. J. F. Marko and E. D. Siggia, *Macromolecules* **28**, 8759 (1995).
34. J. A. Holbrook, O. V. Tsodikov, R. M. Saecker and M. T. Record, *J. Mol. Biol.* **310**, 379 (2001).
35. R. T. Dame and N. Goosen, *FEBS Lett.* **529**, 151 (2002).
36. C. Bouchiat and M. Mézard, *Eur. Phys. J. E Soft Matter* **2**, 377 (2000).
37. J. F. Marko, Introduction to Single-DNA Micromechanics, to appear in *"Proceedings of the Les Houches Summer School 2004, Session LXXXII"*.
38. J. D. Moroz and P. Nelson, *Proc. Natl. Acad. Sci. USA* **94**, 14418 (1997).
39. C. Bouchiat and M. Mézard, *Phys. Rev. Lett.* **80**, 1556 (1998).
40. C. Bouchiat, M. D. Wang, J.-F. Allemand, T. Strick, S. M. Block and V. Croquette, *Biophys. J.* **76**, 409 (1999).
41. J. F. Marko and E. D. Siggia, *Macromolecules* **27**, 981 (1994).
42. D. M. Gowers and S. E. Halford, *EMBO J.* **22**, 1410 (2003).
43. M. Geanacopoulos, G. Vasmatzis, V. B. Zhurkin and S. Adhya, *Nat. Struct. Biol.* **8**, 432 (2001).
44. H. Y. Wu, S. Shyy, J. C. Wang and L. F. Liu, *Cell* **53**, 433 (1988).