DNA condensation by TmHU studied by optical tweezers, AFM and molecular dynamics simulations

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Abstract The compaction of DNA by the HU protein from *Thermotoga maritima* (TmHU) is analysed on a single-molecule level by the usage of an optical tweezers-assisted force clamp. The condensation reaction is investigated at forces between 2 and 40 pN applied to the ends of the DNA as well as in dependence on the TmHU concentration. At 2 and 5 pN, the DNA compaction down to 30% of the initial end-to-end distance takes place in two regimes. Increasing the force changes the progression of the reaction until almost nothing is observed at 40 pN. Based on the results of steered molecular dynamics simulations, the first regime of the length reduction is assigned to a primary level of DNA compaction by TmHU. The second one is supposed to correspond to the formation of higher levels of structural organisation. These findings are supported by results obtained by atomic force microscopy.

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Abbreviations

TmHU histone-like protein from *Thermotoga maritima*;
bp basepairs;
Da Dalton

1 Introduction

The prokaryotic genome is not imbedded in a cellular compartment like the eukaryotic nucleus, but is arranged in a highly compacted way in a particular region of the cytoplasm called the nucleoid. The mechanisms that account for this compaction are DNA supercoiling, macromolecular crowding and the action of nucleoid-associated proteins, but their relative contributions are still unclear [1]. The genome of the hyperthermophilic bacterium *Thermotoga maritima*, which appears as one circular chromosome, amounts to 1,860,725 bp corresponding to a circumference of $\sim 630 \mu\text{m}$ [2]. A single *T. maritima* organism has a rod-like shape with an average length of $5 \mu\text{m}$ [3], implying a micrometre-sized nucleoid. These dimensions underline the importance of powerful mechanisms of compaction that make such a dense packing possible but yet ensure the flexibility to accomplish transcription and replication.

About a dozen proteins, like the histone-like nucleoid structuring protein (H-NS), heat unstable protein (HU), DNA protection during starvation (Dps) and integration host factor (IHF) associate with the nucleoid of the mesophilic bacterium *Escherichia coli* [1]. Similar proteins are believed to play a role in other organisms. However, little is known about the compaction and organisation of the DNA in *T. maritima*, since it does not encode homologs of IHF, Dps or H-NS [4]. The histone-like protein HU, which is nearly ubiquitous in all bacteria, has been found to alter the structure of DNA by introducing kinks and bends [5, 6]. The HU protein of *E. coli* is quite well characterised. It acts on the DNA in an opposed modality: the complex forms stiff filaments without condensation at high protein concentrations whereas at low concentrations the DNA is compacted to 50% of its initial length [7]. Despite the increasing knowledge about DNA compaction in bacteria, it has to be noted that the nature of the contribution of HU proteins to nucleoid structure is still under discussion [8]. Recent reviews by Dame et al. [1] and Luijsterburg et al. [9] describe in detail the current state of knowledge on the contribution of nucleoid-associated proteins to the nucleoid structure. It is supposed that DNA-bending proteins, to which HU proteins belong, do not only play a role in compacting DNA but also have the ability to remodel bacterial chromatin by dislocating the arrangement of loops. However, none of the nucleoid-associated proteins is solely responsible for the organisation of bacterial chromatin. This is corroborated by the fact that bacterial cells that are deficient in one of the nucleoid-associated proteins were usually found to be viable, which suggests that the function of one of those proteins can be taken over by another. Thus, the organisation of bacterial chromatin is rather a matter of an interplay of different proteins that co-operate, compete and also antagonise each other.

Here we concentrate on the role of the HU protein of *T. maritima* (TmHU). In its functional form TmHU is composed of two identical subunits with a molecular weight of 9,994.0 Da each. These form a core part with two protruding beta-ribbon arms [5]. TmHU

has the ability to tightly bind DNA ($K_d = 5.6$ nM) without a detectable sequence specificity [10]. X-ray studies and structure modelling show that the DNA is bent around the protein dimer with an angle of $\sim 160^\circ$ with the emerging beta-ribbon arms embracing the DNA. The binding leads to a compaction of the double-stranded DNA [5, 11]. Furthermore, TmHU is extraordinarily stable at high temperatures as thermal denaturation of the secondary structure occurs well above 96°C and is nearly completely reversible. One suggested major task of TmHU is to protect the DNA of *T. maritima* against thermal denaturation [5]. With an optimum growth temperature of about 80°C , the organism is adapted to its habitat on the geothermally heated sea floors in Italy and the Azores [3].

Optical tweezers provide an extraordinary technique for the investigation of the interactions between biological macromolecules on a single-molecule level [12] as they allow for positioning a micron-sized particle with nanometre resolution and measuring the forces acting on it with an accuracy of ~ 50 fN. Using this technique, the elastic properties of double-stranded DNA have been characterised [13–15] as well as their dependence on the ionic conditions [16]. Furthermore, the interaction of DNA molecules with binding partners like transcription factors [17] or intercalating dyes [18] has been studied. Other work investigates antigen–antibody interaction [19], RNA folding [20, 21] and individual steps of single-kinesin motor proteins [22, 23]. Wen et al. observed the translation at a ribosome on the level of single codons [24]. Particularly the investigation of architectural proteins binding to DNA is an ongoing topic of special interest. By stretching single chromatin fibres, histone proteins can be dissociated from the DNA. The force–distance dependence shows a saw-tooth-pattern whose distinct events are interpreted in terms of single DNA/histone complexes being destabilised [25–27]. Dame et al. observed directly the bridging of two DNA duplexes by H-NS employing a quadrupole trap in order to clamp two DNA duplexes [28].

The interaction of TmHU with a single double-stranded DNA molecule has recently been investigated by optical tweezers [29]. We observed a shortening of the contour length accompanied by a steep force increase up to ~ 30 pN where the reaction was inhibited. Investigating the effects of varying protein concentration yielded information about the kinetics of the reaction [30]. As a further refinement, our set-up is now furnished with a force feedback loop, allowing us to keep the force constant during the experiment. In the present work, the condensation reaction under the action of a constant force is investigated at different force levels, amongst others with regard to the question of whether the final TmHU/DNA complex builds up in different organisational levels. Furthermore, the influence of the protein concentration is analysed. The results are confirmed by measurements with atomic force microscopy (AFM). Finally, steered molecular dynamics (SMD) simulations provide a molecular view of the disassembly of a single TmHU/DNA complex when it is stretched.

2 Materials and methods

2.1 Optical tweezers setup

The force on a single DNA molecule is set and controlled by optical tweezers with video-based force feedback. We create the optical trap with a diode-pumped Nd:YAG laser (1,064 nm, 1 W, LCS-DTL 322; Laser 2000, Wessling, Germany) coupled into a commercial microscope (Axiovert S 100 TV, Carl Zeiss, Jena, Germany). A PID-controller stabilises the

power output of the laser. After passing an optical isolator a quarter-wave plate is employed to produce circular polarised light to exclude effects due to reflection differences between the p and s parts of the laser light. The beam is expanded and coupled into the back aperture of the microscope objective (Plan-Neofluor 100 × 1.30 Oil, Carl Zeiss, Jena, Germany). Video imaging and optical position detection are accomplished by a charge-coupled device digital camera (Dalsa, Munich, Germany). During the measurements we acquire images with a camera at a frequency of up to 60 frames per second. The microparticle position is extracted on-line from the obtained images via image analysis as described by Otto et al. [31]. The precision in the position determination in the relevant axes x and y is ± 2 nm. In order to hold the force at a constant level during the experiment, a LabView-based PID-control (force clamp) with a regulation frequency of up to 30 Hz is employed. The optical stage can be positioned in three dimensions with nanometre resolution using piezoactuators (P-5173CD, PI; Karlsruhe, Germany). The sample cell with a volume of ~ 300 μl consists of a stainless steel corpus, which is covered at the top and at the bottom by glass coverslips. A custom-made micropipette with an inner diameter at the tip of < 1 μm is inserted into the chamber to hold one microparticle by capillary action. Additional inlets and outlets enable flushing the cell with buffer or protein solution using a syringe pump. The whole experimental set-up is situated in a temperature-controlled (± 1 K) room.

2.2 Sample preparation

All measurements are carried out in 10 mM phosphate buffer (pH 7.0) with a sodium chloride concentration of 20 mM. For the experiments, a DNA molecule is immobilised between two polystyrene microparticles (diameter 2.0 μm) via streptavidin/biotin and digoxigenin/anti-digoxigenin (anti-DIG) interaction. The double-stranded 4,000-bp long DNA is amplified from the plasmid pET28a+ via polymerase chain reaction (PCR) and labelled with digoxigenin and biotin, respectively, at their termini via 5'-end modifications of the primers (Metabion, Matinsried, Germany) that are used for PCR. The obtained DNA molecules are immobilised on the surface of streptavidin-coated microparticles (diameter 2.0 μm , Polysciences Europe, Eppelheim, Germany) with a density of 150 molecules per particle.

The anti-DIG covered particles are made by coating carboxylated microparticles (G. Kisker GbR, Steinfurt, Germany) with anti-DIG antibodies. The preparation of the microparticles and the synthesis and immobilisation of the DNA molecules are carried out according a protocol developed by Salomo et al. [29].

2.3 Experimental procedure

In order to establish a single DNA duplex, the particle with anti-DIG antibodies is captured by the optical trap and brought in contact with the tip of the micropipette where it is immobilised by capillary forces. Afterwards a second particle (coated with streptavidin and covered with DNA) is trapped by the optical tweezers and brought into the immediate neighbourhood of the anti-DIG-particle so that a visible distance between the particles remains. The binding is established when the particle in the optical trap is slightly pulled out of the focus while retracting the particle at the micropipette. To ensure that only one chain is linked between the two particles, a force–distance dependence of unmodified DNA is recorded (data not shown). After these initial tests, the TmHU protein is flushed into the

sample cell with varying concentrations, while the force on the double-stranded DNA is held constant. The condensation of the DNA is recorded by on-line video detection.

2.4 Atomic force microscopy

TmHU–DNA binding reactions were performed on the mica surface while still in solution. Samples for AFM imaging were prepared by depositing 5 μl of a solution containing 5 ng of DNA, 15 mM MgCl_2 and phosphate buffer with 20 mM NaCl (pH 7.0) onto flat freshly cleaved mica. After 2 min, another droplet of 5 μl was added to the one on the surface containing the desired TmHU concentration and 15 mM MgCl_2 in the same buffer. The protein concentration values (20–100 $\mu\text{g/ml}$) refer to the one in the final volume (10 μl). Due to the short incubation time evaporation was not an issue. Various incubation times after mixing were tested, however, and no notable difference for longer times than 5 min was observed. After the binding reaction, the sample was washed with DI-filtered water and blown dry in a gentle stream of nitrogen gas. Samples were imaged at 1.95 nm per pixel resolution in air at room temperature and humidity using tapping mode imaging (cantilever stiffness between 27 and 59 N/m, Nanosensors, Neuchatel, Switzerland) to minimise sample-tip interactions with a Dimension 3000 system with a Nanoscope IIIa controller (Veeco Instruments, Santa Barbara, CA, USA). Image processing including flattening was done using WSxM freeware (www.nanotec.es). AFM imaging was always performed right after the mica had been dried. All experiments were repeated at least three times with new samples on different days to check for reproducibility.

2.5 Molecular model and simulation setup

The SMD³⁴ simulation is based on the crystal structures of a single TmHU (PDB entry: 1B8Z), which only contains the residues 1 to 52 and 76 to 90 [32]. The missing beta-ribbon arms were added by homology modelling based on the related HU protein of *Anabaena* (AHU, PDB entry: 1P71) [6]. The latter was resolved including the beta-ribbon arms and a pre-bended 19-bp long double-stranded DNA. The DNA from the AHU structure was extended to a total number of 35 bp with the sequence GCCAAAAAATGCAACAATTTGTTGCATTTTTTGGC. The middle part of this sequence results from the crystal structure while the rest of the sequence was taken from a computer model of an equilibrated DNA strand added in a quasi-symmetric fashion.

Both the protruding beta-ribbon arms and the DNA, were then combined with the crystal structure of TmHU. Together with approximately 26,500 water molecules, the system fills a rectangular box of about $6 \times 18 \times 8.5 \text{ nm}^3$. To neutralise the system, 42 Na^+ ions were added.

The simulation is performed using NAMD 2.6 [33] together with the CHARMM27 [34] force field, a time step of 1 fs and the PME method for calculating the long-range electrostatic interactions. After an energy minimisation, an equilibration run has been performed at 300 K in a NpT ensemble for 2 ns. Already after 500 ps no large change of the RMSD remains visible. The subsequent 30 ns stretching simulation of the DNA/TmHU complex took place in an NVT ensemble. During this process the protein was kept in place by a cylindrical potential encompassing the helical body only, i.e. the arms of the protein were not constrained. This additional potential is necessary in order to keep the protein in place while stretching the DNA. At the same time the TmHU is kept as flexible as possible

during the simulation. Both free ends of the DNA were pulled away from the protein (in $\pm x$ direction) with a spring constant of 70 pN/nm and a constant velocity of 0.5 m/s. It should be pointed out that the stretching velocity in the experiment is only 5 nm/s. Using the latter velocity would lead to simulation times for the complete process that cannot be covered by current atomistic simulations.

3 Results

In Fig. 1, we show three typical results for the condensation of DNA by TmHU. The DNA with a length of 4,000 bp ($\cong 1.36 \mu\text{m}$) is held with a constant force of 2 pN while the protein solution at a concentration of 100 $\mu\text{g/ml}$ is flushed into the sample cell. The force–distance curve of the DNA shows, according to the wormlike chain model, a steep slope for forces higher than 1–2 pN [15]. Thus, every change in force due to protein bindings results in an easily detectable change in length of the DNA. Therefore, a level of 2 pN has been determined as the lowest value to enable the feedback loop to hold the force stable at a preset value. The measurement is carried out while flushing the cell with protein solution perpendicular to the spanned DNA. The influence of the resulting drag force is found to be negligible due to the design of our flow cell with a height of approximately 2 mm, where the inlets and outlets are on a different level than the trapped colloid.

As seen in Fig. 1, the condensation reaction starts after a lag-time of several seconds. The moment of $t = 0$ was located about 10 s before the reaction starts, as the time delay between

Fig. 1 Condensation reaction at 2 pN. Three typical results for the condensation of DNA by TmHU at a force level of 2 pN with a protein concentration of 100 $\mu\text{g/ml}$ (blue, red, green) and a control trace of DNA in the absence of TmHU (orange) are displayed. The DNA duplex is held at a constant force level (± 0.4 pN) while the protein solution is flushed in. The change in length of the DNA under the action of TmHU is recorded, which is defined as the difference to the plateau at $t = 0$ –10 s. The moment of $t = 0$ was located about 10 s before the reaction starts. We distinguish two regimes during the reaction, the initial one, where shortening happens linearly with a rate of (70 ± 30) nm/s until a shortening of (0.77 ± 0.12) μm is reached, and the subsequent condensation (for $t > 10$ s) that proceeds at a much slower rate and is less continuous. The reaction comes to a halt at approximately 1 μm shortening. *Inset* shows the scheme of the experiment. (a) A single double-stranded DNA is immobilised between two microparticles. One of them is held by the optical trap with a constant force while the other is fixed at the micropipette tip. (b) After flushing the protein solution into the sample chamber the condensation of the DNA can be observed

starting the syringe pump and the beginning of the condensation varies significantly due to our setup and the flow cell design. The shortening of the DNA is defined as the change of the end-to-end distance compared to the plateau at $t = 0$ –10 s. We distinguish two regimes during the reaction. Initially, the reduction in length happens fast with a rate of (70 ± 30) nm/s until a shortening of (0.77 ± 0.12) μm is reached. The subsequent condensation (for $t > 10$ s) proceeds at a much slower rate and less continuously until the reaction comes to a halt at approximately 1 μm shortening, which corresponds to $\sim 70\%$ of the initial end-to-end distance of the DNA. All measurements were performed with a new pair of microparticles.

Figure 2 shows the shortening of the DNA due to the binding of TmHU as a function of the applied force. The condensation was carried out at a protein concentration of 100 $\mu\text{g}/\text{ml}$. We detect shortening of the DNA up to a pre-stretching of 40 pN. The maximal change in length decreases exponentially from ~ 1 μm (2 pN) to ~ 60 nm (40 pN; Fig. 2, inset). The proceeding of the reaction depends on the applied force and, in contrast to the case for 2 pN, above 10 pN no slow shortening is observed.

Furthermore, the influence of TmHU concentration on the DNA condensation at a constant force is investigated. Figure 3 depicts typical results for TmHU concentrations ranging from 20 to 250 $\mu\text{g}/\text{ml}$ at a force level of 2 pN. The rate of condensation is defined as the change in DNA length per time between the plateau at $t = 0$ s and the level of 0.5 μm . This level is reached in all experiments at forces lower than 10 pN. The TmHU concentration has a significant impact on the rate of DNA condensation. At concentrations above 50 $\mu\text{g}/\text{ml}$, the DNA shortening up to 0.77 μm is a continuous process with a rate of (70 ± 30) nm/s. At lower protein concentrations the condensation advances much slower and finally reaches a minimum rate of (10 ± 5) nm/s at 20 $\mu\text{g}/\text{ml}$ (Fig. 3). Furthermore, the condensation process is less continuous, and is interrupted by pauses. Both the total reduction in length and the rates are shown as a function of protein concentration in the insets of Fig. 3. The dots indicate the averages over all data points at the respective concentration. For concentrations below 20 $\mu\text{g}/\text{ml}$ no shortening of the DNA could be observed on the time scale of several minutes. During the whole time protein solution was flushed in continuously. Working in continuous flow ensures that non-specific adhesion

Fig. 2 Force dependence of the condensation reaction. The TmHU-induced condensation of the DNA at different force levels is displayed. With increasing force, the shortening decreases significantly, until at 40 pN almost no condensation is taking place. In contrast to the case for 2 pN, above 10 pN no slow shortening is observed. *Inset* shows mean shortening at different forces. The *error bars* indicate minimal and maximal values

Fig. 3 Concentration dependence of the condensation reaction. The condensation of DNA by TmHU at a force of 2 pN is compared for concentrations between 250 and 20 $\mu\text{g/ml}$. At smaller protein concentrations the reduction in length proceeds slower and less linearly. *Insets* show the mean values of at least five single measurements of (a) the total reduction in length and (b) the condensation rate to a shortening of 0.5 μm . The error bars are given by minimal and maximal values. Both rate and final extension increase with increasing concentration. At 100 $\mu\text{g/ml}$, a saturation is observed

to the sample chamber does not inhibit the reaction. However, it has to be noted that the applied tension of 2 pN might be sufficient to impede detectable binding.

Additionally, the TmHU/DNA complex was investigated by AFM. A distinct dependence of the shape of the protein complexes on the protein concentration is found. Figure 4 compares bare DNA (250 bp) with the TmHU/DNA complex at TmHU concentrations of 20 and 100 $\mu\text{g/ml}$. The bare DNA shows, with the mean end-to-end distance of 70 nm,

Fig. 4 Condensation of DNA (250 bp) by TmHU observed by AFM at TmHU concentrations of 0 mg/ml, 20 $\mu\text{g/ml}$ and 100 $\mu\text{g/ml}$. The results presented here are representative for the 30–100 individual complexes that were analysed for each concentration. *Insets* show zooming in and height profile along the blue line. *Left* AFM image of double-stranded DNA (250 bp). The DNA shows an extended shape with mean end-to-end distance of 70 nm. The height profile displays a value around 0.5 nm. *Middle* at 20 $\mu\text{g/ml}$, the complexes still show an extended shape with a mean end-to-end distance of 42 nm. However, the height of the complex is here almost 2 nm. *Right* the structure of the complexes at 100 $\mu\text{g/ml}$ is highly condensed and has a globular shape with a diameter of 38 nm

an extended shape and a height around 0.5 nm. At a TmHU concentration of 20 $\mu\text{g/ml}$ the complex height is increased to ~ 2 nm. Although the end-to-end distance is reduced to 42 nm on average, the complex still shows an extended shape. At the higher concentration of 100 $\mu\text{g/ml}$, condensed complexes with globular shape and a diameter of 38 nm are formed, which is another clear indication for a second organisational level.

In addition to the experimental observations, we present here the results of SMD simulations of the stretching of a single TmHU/DNA complex. Salomo et al. observed events of the size of 2–3 nm while stretching a DNA duplex after incubation with TmHU. These events were assigned to the disruption of single TmHU proteins from DNA by comparison with a structural model [29]. SMD simulations allow us to gain valuable insight into the complex dynamics of this unbinding process. As shown in Fig. 5A, forces pointing into opposite directions were applied to both DNA tails contacting a single TmHU protein during a constant velocity simulation. In such a simulation a certain set of atoms is harmonically constrained to a reference point that moves with constant velocity. The DNA has a kinked conformation and is bound to the helical body of the protein (Fig. 5A). From those binding positions, one obtains an effective binding length of 22 bp for the DNA embracing the protein. One can subdivide the simulation into three parts. During the first third of the simulation the force increases almost linearly with the distance between the DNA tails until a force of roughly 180 pN is reached. The DNA remains bound to the helical body of the protein. In the second part of the simulation the unbinding of the DNA from the helical body of the protein takes place. As shown in the graph of Fig. 5B, this may happen in an asymmetric fashion. The thermal fluctuations also present in the simulation

Fig. 5 Force–distance dependence of the SMD-simulated stretching of the TmHU–DNA complex. The 35 bp DNA is stretched with a velocity of 0.5 m/s with a force constant of 70 pN/nm. The snapshots *A–D* show the rupture of bonds between the DNA and the protein. The corresponding parts of the force–distance trace are indicated. *A* Snapshot at $t = 7.5$ ns. The arrows indicate the two resulting forces pointing in opposite directions. The force increases almost linearly with the distance of the DNA ends to the protein. The DNA sticks to both sides of the protein. *B* Snapshot at $t = 11.0$ ns. The DNA is disrupted from the left side of the helical body of the protein, which leads to an increase in distance of about 2 nm. Simultaneously, an unwinding of both DNA tails takes place, which yields an additional extension of ~ 1 nm each. *C* Snapshot at $t = 20.25$ ns. At around 500 pN, the right part of the DNA unbinds from the body of the protein and relaxes for another 2 nm. *D* Snapshot at $t = 25.0$ ns. Both DNA ends are released from the protein and are stretched further

comprise a stochastic element and determine, together with the initial conditions of the simulation, whether the DNA disrupts first only from one side of the protein or from both sides at the same time. For the present simulation, the part of the DNA on the right hand side of the protein remains in contact, while the DNA part on the other side detaches from the protein (Fig. 5B). During this disruption event the distance between the left DNA tail and the protein increases by about 2 nm. At the same time an unwinding of both DNA tails takes place resulting in an additional extension. The unwinding is possible since the forces are only applied to the centres of mass of certain atom selections and is indeed the reason for the first plateau in the force-distance curve between 2.7 and 3.7 nm for the DNA part pulled in the positive x direction. Furthermore, one can see in the force-distance curve as well as in Fig. 5C that the interaction between the DNA and the protein on the right hand side is so strong that the force increases up to values of 500 pN. As expected for these large forces [35], an unwinding of the DNA together with a structural change to a ladder conformation is observed. The reason for this tight binding between DNA and protein is strongly related to the residues LYS41, GLY46, LYS80 and LYS83 of the protein, which interact with the DNA backbone through hydrogen bonds. The residue LYS83 is initially positioned parallel to the backbone of the DNA and in this way stabilises the binding to the DNA. Towards the end of the simulation this part of the DNA also unbinds from the body of the protein and relaxes again for another 2 nm as can be identified by the second plateau for the DNA tail pulled in the $+x$ direction between 4.6 and 6.6 nm (Fig. 5D). This stretching corresponds to the length between the initial binding position of the beta-ribbon arms at the helical body of the protein and the midpoint between the arms. Prior to the just described rupture event, the structure of the DNA between the two protruding beta-ribbon arms is not degenerated. In the final part of the simulation both DNA tails are unbound from the helical body of the protein and stretched further. At this point the two curves in Fig. 5 again show a similar behaviour with increasing force. Moreover, the part of the DNA between the arms is stretched further in a similar fashion. One should note that also in the final part of the simulation the DNA is still connected to the protein at the midpoint between the arms.

4 Discussion

The condensation reaction at 2 pN is found to take place in two regimes. The first one leads to a shortening of 0.77 μm , after which the compaction continues much slower to the final length reduction of 1 μm . From the literature as well as from our present SMD simulation we obtain quantitative information concerning the binding length and the reduction in length due to the binding of one protein to DNA. Therefore, it is possible to estimate a shortening which can then be related to the observed value, in order to assign the regimes to a certain binding mode. It is known from X-ray studies and structural modelling that, as depicted above and displayed in Fig. 5, the DNA is bent around a TmHU protein in an arc [5, 11]. In the common model for a complex with more than one protein binding to DNA, the proteins arrange antiparallel, partly sandwiching the DNA as shown in Fig. 6 [4, 6, 29]. This arrangement allows overcoming a kinetic delay caused by the energetic effort to bend the DNA [29]. The initial state of the SMD simulation allows the precise determination of the length of the DNA that is bound to the protein to be 22 bp. This value agrees quite nicely with the data from the literature where the effective binding length in such a complex was determined experimentally to be 26.8 bp [4], and estimated from the structural model to be 24 bp [29], respectively. In the SMD simulation on DNA with a specific sequence

Fig. 6 Visualisation of the supposed structural formation of the TmHU/DNA complex. The condensation starts with a primary TmHU binding until a shortening of around $0.77\ \mu\text{m}$ is reached. Henceforward, the final complex builds up in higher levels of structural organisation. The total reduction in length compared to the extension of the bare DNA reaches around $1\ \mu\text{m}$, which corresponds to about 70% of the initial DNA length

we observe that a one-sided unbinding of DNA and protein causes a lengthening of the end-to-end distance of the DNA of at least 2 nm. With this finding it is possible to assign the experimentally observed disruption events of 2–3 nm from recent experiments [30]. Due to the high forces the unbinding process during the simulation is a combination of an unwinding of the DNA tails and a detaching from the protein. The observed reverse order of unwinding and detaching for pulling in the $+x$ and $-x$ directions is most likely a consequence of the initial setup and conditions together with the thermal/stochastic fluctuations resulting in the asymmetric force profiles in Fig. 5. In the simulation forces of up to 500 pN are reached, which is one order of magnitude higher than encountered under experimental conditions. Concerning this matter one has to keep in mind that, in contrast to the latter one, the simulation proceeds with a pulling rate of 0.5 m/s far out of equilibrium. This causes the observed high forces as it is known from the literature that the unfolding force depends linearly on the logarithm of the pulling speed [36–38]. A similar problem is, for example, faced in the unfolding process of titin sub-domains [39, 40], and in dynamic force spectroscopy studies of DNA [35, 36]. Because of those large forces in our simulation, the DNA parts, which are not attached to the protein, start unwinding before the connections to the protein break. This is not expected to happen in the optical tweezers experiment due to the much smaller forces involved. We also would like to note that the flexibility of a DNA strand exhibits a dependence on the sequence [41, 42]. Although no sequence specificity of TmHU binding is detectable experimentally [10], it is conceivable that changing the DNA sequence might influence the unbinding process.

With the knowledge of the effective binding length found in the SMD calculations (22 bp) and the length change due to the disruption of one protein of at least 4 nm, one can now go back to the model for a complex with more than one TmHU protein binding to DNA and calculate the expected TmHU-induced shortening ΔL for a saturated double-stranded DNA (initial length, 4,000 bp $\cong 1.36\ \mu\text{m}$):

$$\Delta L = 4,000\text{ bp}/22\text{ bp} \times 4\text{ nm} \approx 0.73\ \mu\text{m}.$$

The value matches within the experimental error with our result at 2 pN and a concentration of 100 $\mu\text{g}/\text{ml}$, where the end-to-end distance is decreased by about $0.77\ \mu\text{m}$. This is remarkable given the different regimes probed by the simulations and measurements.

However, together with the second regime, a total shortening of approximately $1\text{ }\mu\text{m}$ has been observed in our experiments. The simple binding model of antiparallel-arranged proteins is not sufficient to explain this finding. Our experimental results imply that in the first regime the DNA is condensed in a linear manner as proposed in the model, corresponding to a primary level of DNA compaction. The additional shortening observed in the second regime suggests that this primary structure of the TmHU/DNA complex is further compacted in a higher level of organisation (Fig. 6). Large rupture events obtained when stretching the TmHU/DNA complex give only indirect indications concerning the existence of such clusters [30]. This is also consistent with the structure observed by AFM measurements at $100\text{ }\mu\text{g/ml}$ (Fig. 4), showing a compacted, globular shape. Elongated filaments as expected if TmHU proteins were bound like beads-on-a-string are not found in this regime. It is supposed that this higher level of organisation depends strongly on the TmHU concentration. For concentrations smaller than $100\text{ }\mu\text{g/ml}$ a lesser reduction in length is observed and the division into two regimes is no longer possible (Fig. 3). The elongated filaments found by AFM measurements at $20\text{ }\mu\text{g/ml}$ support this assumption. Though the system is in this case constricted to two dimensions and short DNA is used (250 bp), the result is in qualitative agreement with the optical tweezers measurement. These results on the concentration dependence are consistent with the observation of Salomo et al. that large rupture events occur only after incubation with a high TmHU concentration of at least $100\text{ }\mu\text{g/ml}$ [30]. Up to now there are no concrete model representations concerning higher-order organisation of DNA by HU proteins and especially by TmHU. It is presumed that the HU/DNA complexes in the case of the HU protein from *E. coli* are not stable and are of a dynamic nature [43]. Therefore, a similar behaviour in the case of the homologous TmHU protein is very likely. This might be related to the observed slow formation of the second phase of condensation.

The measurements at different force levels show a distinct influence on the condensation of the DNA. At a force of 10 pN the reduction in length of the DNA is only $\sim 0.5\text{ }\mu\text{m}$. Furthermore, the second regime is not observed, implying that the higher level of structural organisation observed at lower forces and in the AFM images can no longer be formed. Astonishingly, compared with the condensation reaction without force clamp that came to a halt at $25\text{--}30\text{ pN}$ [29], a shortening against a pre-stretching of up to 40 pN is observed. The mechanism for protein binding differs for conditions with and without force clamp since in the case of increasing force the DNA is already covered with proteins when 25 pN are reached.

5 Conclusions

The condensation of double-stranded DNA by TmHU under constant force is investigated by usage of an optical tweezers-assisted force clamp. At forces of up to 5 pN , the end-to-end distance is reduced from about 1.36 to $\sim 0.36\text{ }\mu\text{m}$ corresponding to 30% of its initial length on a time scale of seconds. This length-reducing reaction can be split into two regimes. Joining data from our SMD simulation and information from the literature, the first regime is assigned to a primary binding of TmHU. The second regime is therefore suggested to correspond to the formation of a higher level of organisation whose existence was already presumed in the literature [30]. In addition, the results from the SMD simulation precisely describe the process of an unbinding forced by stretching the DNA. This yields a detailed

molecular explanation for the rupture events which were recently observed experimentally [30].

The final DNA length of $\sim 0.36 \mu\text{m}$ is comparable to the mean end-to-end distance of $\sim 0.33 \mu\text{m}$ of a free DNA molecule in the same buffer solution (persistence length, $\sim 40 \text{ nm}$). Hence, the effect of TmHU binding to DNA is much stronger compared to that of *E. coli* HU. In the latter case the DNA duplex is compacted to about 50% of its initial length at a force of 1 pN on a time scale of minutes [7]. This leads to the conclusion that the TmHU protein condensates DNA much faster. Therefore, TmHU features not only a higher affinity to DNA but also a faster on-rate. Furthermore, one has to take the fact into account that no homologs of proteins like IHF or H-NS are found in *T. maritima*, though they are abundant in mesophilic bacteria like *E. coli*. All these facts together show once more the crucial role of TmHU in the nucleoid organisation and in maintaining genomic DNA integrity in extreme environments at 80°C [4, 5, 10].

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