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Force-dependent melting of supercoiled DNA at thermophilic temperatures

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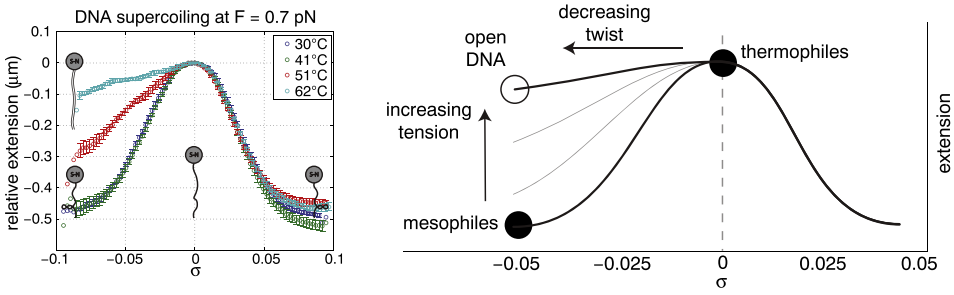
HIGHLIGHTS

- Temperature dependence of force-induced DNA melting on supercoiled templates.
- High-temperature single-molecule magnetic tweezers assay
- Thermophilic temperatures decrease critical tension at which DNA melts.
- Mesophilic and thermophilic strategies for DNA opening may differ.
- Estimation of *in vivo* DNA tension

GRAPHICAL ABSTRACT



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ABSTRACT

Local DNA opening plays an important role in DNA metabolism as the double-helix must be melted before the information contained within may be accessed. Cells finely tune the torsional state of their genomes to strike a balance between stability and accessibility. For example, while mesophilic life forms maintain negatively superhelical genomes, thermophilic life forms use unique mechanisms to maintain relaxed or even positively supercoiled genomes. Here, we use a single-molecule magnetic tweezers approach to quantify the force-dependent equilibrium between DNA melting and supercoiling at high temperatures populated by Thermophiles. We show that negatively supercoiled DNA denatures at 0.5 pN lower tension at thermophilic vs. mesophilic temperatures. This work demonstrates the ability to monitor DNA supercoiling at high temperature and opens the possibility to perform magnetic tweezers assays on thermophilic systems. The data allow for an estimation of the relative energies of base-pairing and DNA bending as a function of temperature and support speculation as to different general mechanisms of DNA opening in different environments. Lastly, our results imply that average *in vivo* DNA tensions range between 0.3 and 1.1 pN.

1. Introduction

DNA metabolism in all organisms is critically dependent on DNA melting. Replication, DNA repair, and transcription all require the opening of the DNA double-helix. However, single-stranded DNA is more susceptible to damage [1–3] and serves as an activator of the ATR-dependent DNA damage checkpoint [4]. Hence, a subtle balance between the double-stranded and open state of DNA must be maintained. This balance is dependent on environmental variables such as salt concentration, pH, torque, force, superhelical density ($\sigma = \Delta Lk/Lk_0$; i.e. the percent of extra or missing turns compared to B-form DNA), and temperature [5–8]. Interestingly, there are differences in superhelical density that correlate with differences in environmental temperature between Mesophiles (20 °C < T < 45 °C; $\sigma < 0$), Thermophiles (45 °C < T < 80 °C; $\sigma \approx 0$), and Hyperthermophiles (80 °C < T < 122 °C; $\sigma > 0$) [9–11]. In addition, hyperthermophilic Archaea have been shown to modulate their superhelical densities in response to either cold or heat shock [12]. These data suggest that DNA metabolism is viable only within a narrow range of

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conditions where the DNA is poised between stability and denaturation. Here, to gain insight into these phenomena, we probe how temperature affects the force-dependent melting of negatively supercoiled DNA using single-molecule magnetic tweezers assays.

Single-molecule DNA manipulation is a powerful technique for probing the details of both the polymer physics of DNA itself as well as for directly observing the activity of enzymes that catalyze reactions on nucleic acids [13–15]. The magnetic tweezers are perfectly suited for studies of DNA melting and denaturation due to the ability to exert both force and torque on constrained DNA molecules [7,8,16–18]. The method depends on tethering DNA between a glass coverslip and a superparamagnetic bead. These linkages contain multiple individual bonds and generate a DNA "tether" that cannot relax torsionally. The beads are manipulated by changing the magnetic field gradient using permanent magnets mounted on a translatable and rotatable stage. The position of the bead is tracked in 3D and is used as a proxy for the position of the end of the DNA, thus allowing for measurements of the end-to-end distance of the DNA as a function of environmental conditions, enzymatic activity, protein binding, force, and torque.

Classic magnetic tweezers experiments showed that rotation–extension plots can be used to describe the relationship between DNA extension (end-to-end distance), force and superhelical density [16,17]. Rotation–extension plots have been used to study DNA buckling [19,20], the balance between supercoiling and DNA denaturation [7,8,16], and protein binding [21]. More specifically, after buckling, DNA responds to increased superhelical density by forming plectonemic writhes in a force-dependent and chiral manner (Fig. 1). Due to the conservation of linking number, extra turns (or links) introduced into the DNA by turning the magnetic bead must be absorbed by a combination of twist (Tw) or writhe (Wr); i.e. $\Delta Lk = \Delta Tw + \Delta Wr$. At low forces, positive and negative turns are absorbed by plectonemic writhes and the extension of the DNA decreases (Fig. 1a, lower curve) [16,17]. However, at higher forces, negative turns are absorbed by a reduction in twist, i.e. DNA melting (Fig. 1a, upper curve) [16,17]. This leads to a continuous reduction in the degree of compaction due to writhing as the force increases (Fig. 1b). The asymmetry is a direct result of the chirality of the double-helix and can be used as a sensitive measurement of DNA denaturation. Recently, this asymmetry has been used to study the details of the melting–writhing equilibrium [7] and its dependence on salt concentration and pH [8]. Here, for the first time, we extend these measurements to high temperature and determine the temperature-dependence of the characteristic force for the transition between writhing and denaturation at thermophilic temperatures.

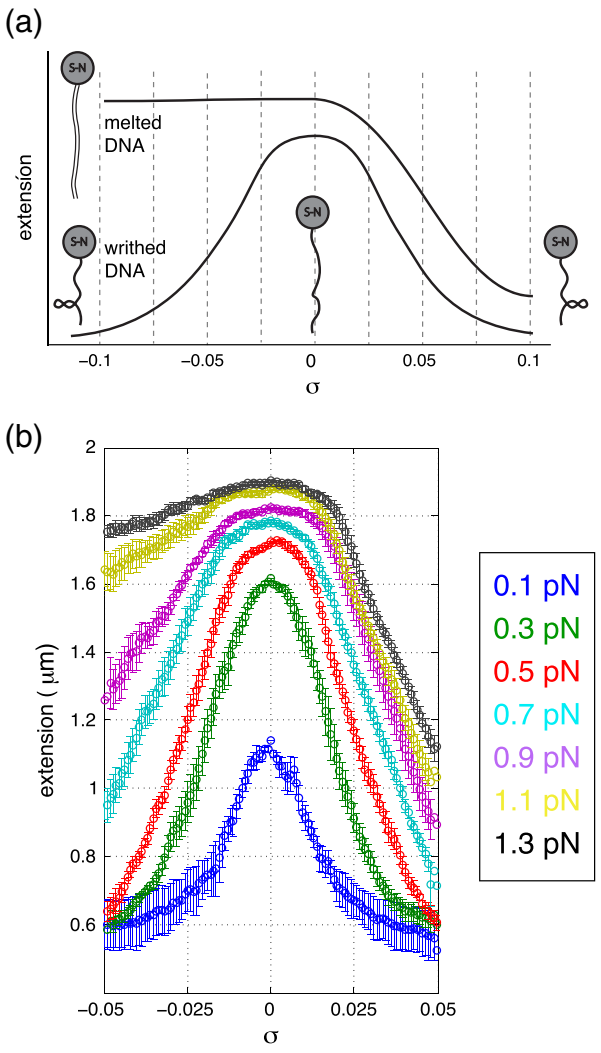

Fig. 1. (a) A schematic showing the force-dependence of DNA extension on superhelical density ($\sigma = \Delta Lk/Lk_0$). At low forces, extension varies symmetrically with increasing positive and negative turns. At high forces, the negative arm becomes flat due to DNA denaturation (melting). (b) Example of the force-dependence of rotation–extension curves for a 6 kb fragment of lambda DNA (bases 16759–22758).

2. Materials and methods

2.1. Flow cell construction

Drilled and un-drilled glass coverslips were sonicated in isopropanol for 15 min and then washed 3 times with water. They were coated with 3 μL of a 1 mg/mL nitrocellulose solution in amyl acetate and left to dry for 10 min. Cut parafilm spacers were sandwiched between a drilled and un-drilled coverslip and melted at 125 °C. After washing with PBS (10 mM NaPO4, 150 mM NaCl, pH 7.4), the cell was incubated with a solution of reference carboxylated polystyrene beads (1:1000 dilution in PBS, Spherotech, CP-15-10, 1.89 μm) for 5 min. After a PBS wash, the cell was incubated with anti-digoxigenin sheep antibodies (Roche, 0.1 mg/mL in PBS) at room temperature for 2 h. Lastly, the cells were passivated with a concentrated solution of BSA (New England Biolabs (NEB), B9001, 10 mg/mL) incubated at 4 °C overnight.

2.2. DNA tether and magnetic bead preparation

DNA tethers were constructed via published protocols [22]. Briefly, 1 kb biotin- and digoxigenin-labeled DNA handles were generated by PCR in the presence of labeled dUTP nucleotides (biotin-16-dUTP or digoxigenin-11-dUTP, Roche). A 2.2 kb core DNA template was also generated by PCR using a DNA template containing the SSVT6 promoter construct from *Methanococcus jannaschii* (gift of D. Grohmann, TU Braunschweig, supplemental information). These fragments were digested with MluI (NEB, R0198) and NheI (NEB, R0198) and ligated with a 4-fold molar excess of handles with T4 DNA ligase (NEB, M0202S). The final 4.2 kb product was gel purified to remove un-ligated DNA handles.

50 μL of streptavidin-coated magnetic beads (Dynabeads MyOne Streptavidin T1, Invitrogen, 1 μm, 65601) were passivated with three 1 mL washes of blocking buffer (PBS, 0.1% Pluronic acid F127, 1 mg/mL BSA) and resuspended in 50 μL of tweezing buffer (PBS, 0.1% Pluronic acid F127, 0.1 mg/mL BSA).

500 nL of 50 pM DNA template was mixed with 10 μL passivated beads and diluted immediately with 90 μL tweezing buffer. The DNA-bound beads were flowed into the flow cell and allowed to incubate for 10 min. Unbound beads were flowed out of the cell with 200 μL at 12 μL/min.