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Molecular Crowding Suppresses Mechanical Stress-Driven DNA Strand Separation

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ABSTRACT

Molecular crowding influences DNA mechanics and DNA - protein interactions and is ubiquitous in living cells. Quantifying the effects of molecular crowding on DNA supercoiling is essential to relating *in-vitro* experiments to *in-vivo* DNA supercoiling. We use single molecule magnetic tweezers to study DNA supercoiling in the presence of dehydrating or crowding co-solutes. To study DNA supercoiling, we apply a stretching force of 0.8 pN to the DNA and then rotate one end of the DNA to induce supercoiling. In a 200 mM NaCl buffer without co-solutes, negatively supercoiled DNA absorbs some of the tortional stress by forming locally melted DNA regions. The base-pairs in these locally melted regions are believed to adopt a configuration where nucleotide base pairing is disrupted. We find that the presence of dehydrating co-solutes like glycerol and ethylene glycol results in further destabilization of base-pairs in negatively supercoiled DNA. The presence of polyethylene glycol, commonly used as crowding agents, suppresses local strand separation and results in plectoneme formation even when DNA is negatively supercoiled. The results presented in this letter suggest many further directions for studies of DNA supercoiling and supercoiled DNA – protein interactions in molecular conditions that approximate *in-vivo* molecular composition.

SIGNIFICANCE

Accurate modelling of DNA mechanics is central to interpreting results of single molecule studies of DNA mechanics and DNA-protein interactions. While the effect of molecular conditions on short and relaxed DNA has been studied, the influence of molecular conditions on DNA supercoiling has not been explored. We present the first single molecule study of DNA supercoiling in presence of crowding and dehydrating co-solutes. We observe that co-solutes can increase or completely suppress stress-driven local strand separation in negatively supercoiled DNA. This change of DNA supercoiling is likely to significantly affect the function of DNA-binding proteins. Our results motivate the need for systematic exploration of DNA supercoiling in presence of co-solutes to accurately relate *in-vitro* DNA-protein interactions to *in-vivo* DNA-protein interactions.

INTRODUCTION

In-vivo, molecular conditions surrounding DNA are varied. In *Escherichia coli* (*E. coli*) cell, DNA is negatively supercoiled and surrounded by 30-40% volume fraction of other organic and inorganic solutes(1, 2). The presence of protein complexes also changes the molecular conditions around DNA. Previous studies have demonstrated that co-solutes affect thermal stability of small and relaxed DNA molecules(3, 4). However, the effect of co-solutes on DNA supercoiling has not been systematically studied.

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The presence of glycerol and ethylene glycol has been demonstrated to reduce the melting temperature of small DNA molecules(5–7). Previous studies have observed that glycerol and ethylene glycol reduce the thermal stability of short DNA molecules because they replace some of the water molecules surrounding the DNA(5, 8, 9). In addition to thermal destabilization, presence of co-solutes can also impact specificity of DNA-protein interactions(10–12).

In the presence of crowding agents like polyethylene glycol (PEG), depending on the size of both PEG and DNA both thermal stabilization and thermal destabilization have been observed(13). Single-molecule magnetic tweezers experiments have also previously observed that DNA can be osmotically compacted by PEG molecules(14–17). The amount of compaction is affected by the counterion valency and the size of PEG molecule(14). Studies of PEG-DNA interactions have also suggested presence of PEG causes a decrease in energy required to form plectonemes(18). PEG molecules can also induce local melting in DNA molecules(19).

In this letter, we investigate the effect of dehydrating and crowding co-solutes on DNA supercoiling. Using single molecule magnetic tweezers(20), we observe that presence of co-solutes can differently affect DNA's response to thermal and mechanical stress. Similar to studies of thermal melting, the presence of glycerol increases local strand separation in mechanically stressed DNA. On the other hand, the presence of ethylene glycol did not significantly affect DNA supercoiling. However, using the polymer polyethylene glycol (PEG) 8000 suppresses base pair separation in negatively supercoiled DNA. The results presented here demonstrate that *in-vivo* DNA supercoiling is influenced by molecular conditions by a combination of chemical and physical effects. Since molecular conditions in living cells are highly crowded, and DNA-protein interactions are sensitive to DNA structure, accurate extrapolation of *in-vitro* DNA-protein interactions studies to *in-vivo* phenomenon requires consideration the influence of co-solutes on DNA supercoiling.

RESULTS AND DISCUSSION

To study the effects of co-solutes on DNA supercoiling, we use single molecule magnetic tweezers and measure the change in extension of DNA due to supercoiling in presence of different co-solutes. We start by measuring the extension-rotation curve in a buffer of 200 mM NaCl. We then flow in a solution of 200 mM NaCl plus co-solutes and compare the extension-rotation curves for the same DNA molecule in different conditions (see additional details in supplementary section S1). The extension-rotation curves are measured with a ~10 kb DNA under a force of 0.8 pN (this force is insufficient to lead to appreciable strand separation under DNA unwinding). In the salt concentration and force condition used here, the extension-rotation curve is asymmetric (blue curves in Fig. 1). For DNA overtwisting (positive supercoiling), DNA absorbs the excess turns by increasing its internal twist, until a critical number of turns is absorbed. Once one passes that critical number of turns, subsequent turns cause the DNA to buckle and form a plectonemic loop. Each subsequent turn causes elongation of the plectoneme and results in a corresponding reduction in DNA extension. In the under-twisting (negative supercoiling) regime, DNA absorbs the applied turns through a combination of local strand separation and plectoneme formation(21).

In the presence of a dehydrating co-solute like glycerol, we observe a decrease in slopes of the extension-rotation curves for both positively and negatively supercoiled DNA. This suggests presence of glycerol results in an increase in local strand separation for both positively and negatively supercoiled DNA (Fig. 1 (B)). Previous studies of DNA response to thermal stress have shown that presence of glycerol reduces the melting temperature of short and relaxed DNA(5–7). Our results indicate a similar destabilization effect of glycerol for DNA under mechanical stress. Similar to previous experiments, we also observe an increase in DNA extension of relaxed DNA due to glycerol(22). Our results suggest that glycerol may alter the base-pair structure of relaxed and supercoiled DNA. Glycerol is a common additive utilized in *in-vitro* experiments of DNA-protein

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interactions. The change in local structure of supercoiled DNA due to glycerol can affect DNA-protein interactions.

To test if DNA local strand separation due to mechanical stress can be exacerbated by other de-hydrating agents, we measured extension-rotation curves in presence of ethylene glycol. Previous studies have observed that presence of ethylene glycol decreases the melting temperature of short DNA molecules(6). In the single molecule magnetic tweezers experiments presented here, we do not observe any increase in local strand separation in presence of ethylene glycol (see Fig 1 (C) and Table 1).

Next, we investigate the effect of polymerized ethylene glycol, polyethylene glycol (PEG), on DNA supercoiling. Previous studies of small DNA molecules have observed that lower molecular weight PEG molecules reduce the melting temperature, while higher molecular weight PEG molecules increase the melting temperature of small DNA(13). Driven by osmotic exclusion, PEG molecules have been observed to induce compaction and condensation in long DNA molecules(14). Higher molecular weight PEG molecules can induce compaction at lower PEG concentrations. Single molecule magnetic tweezers have previously observed salt dependent compaction of relaxed DNA in the presence of PEG(14). The presence of a crowding agent has been shown to enhance the activity of DNA binding proteins. These studies indicate that crowding can play an important role in protein activity(17, 23); however, the effect of crowding on supercoiled DNA has not been studied.

Using single molecule magnetic tweezers, we observe that presence of PEG 8000 suppresses local strand separation in negatively supercoiled DNA. The asymmetry in the extension-rotation curve can be used to estimate the suppression of local strand separation in supercoiled DNA due to PEG 8000. Adding 5% and 10% wt./v PEG 8000 to 200 mM NaCl buffer decreases the asymmetry in the extension-rotation curve, suggesting that helix opening is suppressed in these conditions. In buffer with 15% PEG 8000, the hat curve is symmetrical indicating that local strand separation is completely suppressed.

One might be tempted to attribute the shorter extension of negatively supercoiled DNA in PEG buffer to compression of plectoneme structure caused by osmotic exclusion. However, if osmotic exclusion was a significant factor in the decrease in extension, we ought to have also observed a similar effect of PEG on positively supercoiled DNA. We also find that addition of PEG results in plectoneme formation at a lower linking number. Generally, plectoneme formation due to torsional stress can be modelled as a competition between twisting and bending. Plectoneme formation at a lower linking number would suggest the effect of PEG is some combination of an increase in tortional stiffness of DNA (increasing the energy required to change the intrinsic twist of DNA) and a decrease in bending stiffness of DNA (decreasing the energy required to bend DNA and form a plectoneme loop).

We can use single-molecule magnetic tweezers to analyze the effect of PEG 8000 on bending stiffness of DNA. We observe a moderate increase in persistence length of relaxed DNA in the presence of PEG (see Supplementary Fig. S1). Our finding of significant extension decreases only for negatively supercoiled DNA, buckling at a lower linking number and an increase in persistence length all indicate that PEG increases the torsional stiffness of DNA. The fact that these effects are absent for similar concentrations of ethylene glycol are consistent with a physical molecular-crowding-based mechanism rather than a chemical interaction one. The increase in torsional stiffness in PEG buffer is also consistent with base pair stabilization.

CONCLUSION

We present the first single molecule analysis of DNA supercoiling in presence of a high concentration of co-solutes. We observe that thermal destabilization of DNA in presence of co-solutes does not necessarily

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evidence mechanical destabilization of DNA in similar conditions. We also observed that local strand separation in negatively supercoiled DNA can be suppressed due to presence of crowding co-solutes like PEG 8000. Our results indicate that relating *in-vitro* studies of DNA-protein interactions to *in-vivo* phenomena requires consideration of the change in DNA mechanics in presence of co-solutes; for example, changes to the mechanical properties of DNA (bending and twisting persistence lengths) will result in different behaviors for enzymes which act to bend, twist, or change linking number of the double helix.

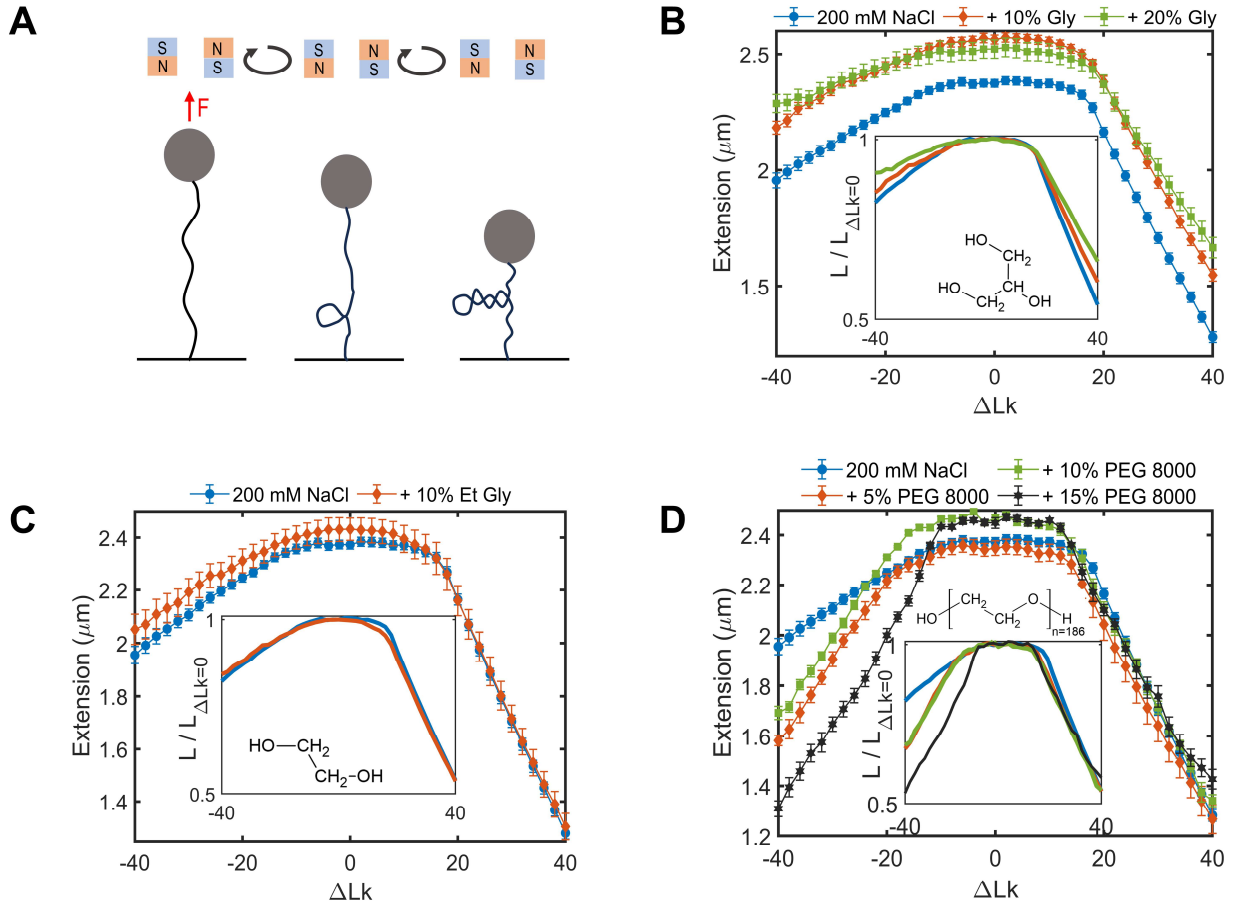


FIG 1. Co-solutes affect DNA supercoiling. (A) Schematic of MT setup. A paramagnetic bead attached to the DNA molecule is used to apply stretching force to the DNA molecule. Buckling can be induced by rotating the paramagnetic bead. (B) Extension- rotation curve of DNA in 200 mM NaCl and 0% (blue), 10% (red) and 20% (green) v/v Glycerol (Gly) buffer. (C) Extension- rotation curve of DNA in 200 mM NaCl and 0% (blue) and 10% (red) v/v Ethylene Glycol (Et Gly) buffer. (D) Extension- rotation curve of DNA in 200 mM NaCl and 0% (blue), 5% (red), 10% (green) and 15% (black) wt./v Polyethylene Glycol 8000 (PEG) buffer. Insets show chemical structure of co-solute, and the extension-rotation curve normalized to the extension of a tortional relaxed DNA in the respective buffer conditions. Error bars represent standard errors. Chemical structures were created using ChemSketch(24).

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AUTHOR CONTRIBUTIONS

P.R.D and J.F.M. designed the project. P.R.D performed experiments and analyzed the data. P.R.D wrote the manuscript. P.R.D. and J.F.M. read and revised the manuscript.

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DECLARATION OF INTERESTS

The authors declare no competing interests.

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Molecular crowding suppresses mechanical stress-driven DNA strand separation

Supplementary Information

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S1. Materials and Methods

DNA Preparation: The DNA substrate was constructed by ligating functionalized digoxigenin and biotin handles (~ 900 bp long) to either end of a linearized pFOS1 plasmid (~ 10 kb). Modified DNA molecules were incubated with the M270 streptavidin-coated magnetic beads. DNA molecules were then incubated in a ~ 40 uL volume flow cell containing anti-digoxigenin functionalized surface. A more detailed protocol of DNA and flow cell construction can be found in Zaichuk et *al*. (1).

Procedure: The flow cell was then washed with > 10* flow cell volume of the standard salt buffer (200 mM NaCl, 20 mM TRIS- pH 7.5, 0.01% Tween 20). Supercoilable DNA molecules were found and verified by negatively supercoiling the DNA molecule under ~1 pN force and then reducing the force to ~0.2 pN. A rapid reduction in DNA extension would be observed if a single supercoilable DNA molecule was attached to the magnetic bead under observation. For the extension-rotation curves, we collected the DNA extension data at a rate of ~70 Hz for 20 seconds at every 2 turns between -40 and +40 turns. The flow cell was then washed with > 10* flow cell volume of the applicable co-solute buffer. The extension-rotation were then repeated for the same DNA molecule allowing measurements of co-solute effects. We collected data for atleast 3 different DNA molecules for each co-solute buffer. Each extension-rotation measurement was repeated at least twice for each molecule.

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S2. DNA bending persistence length and contour length in presence of co-solutes

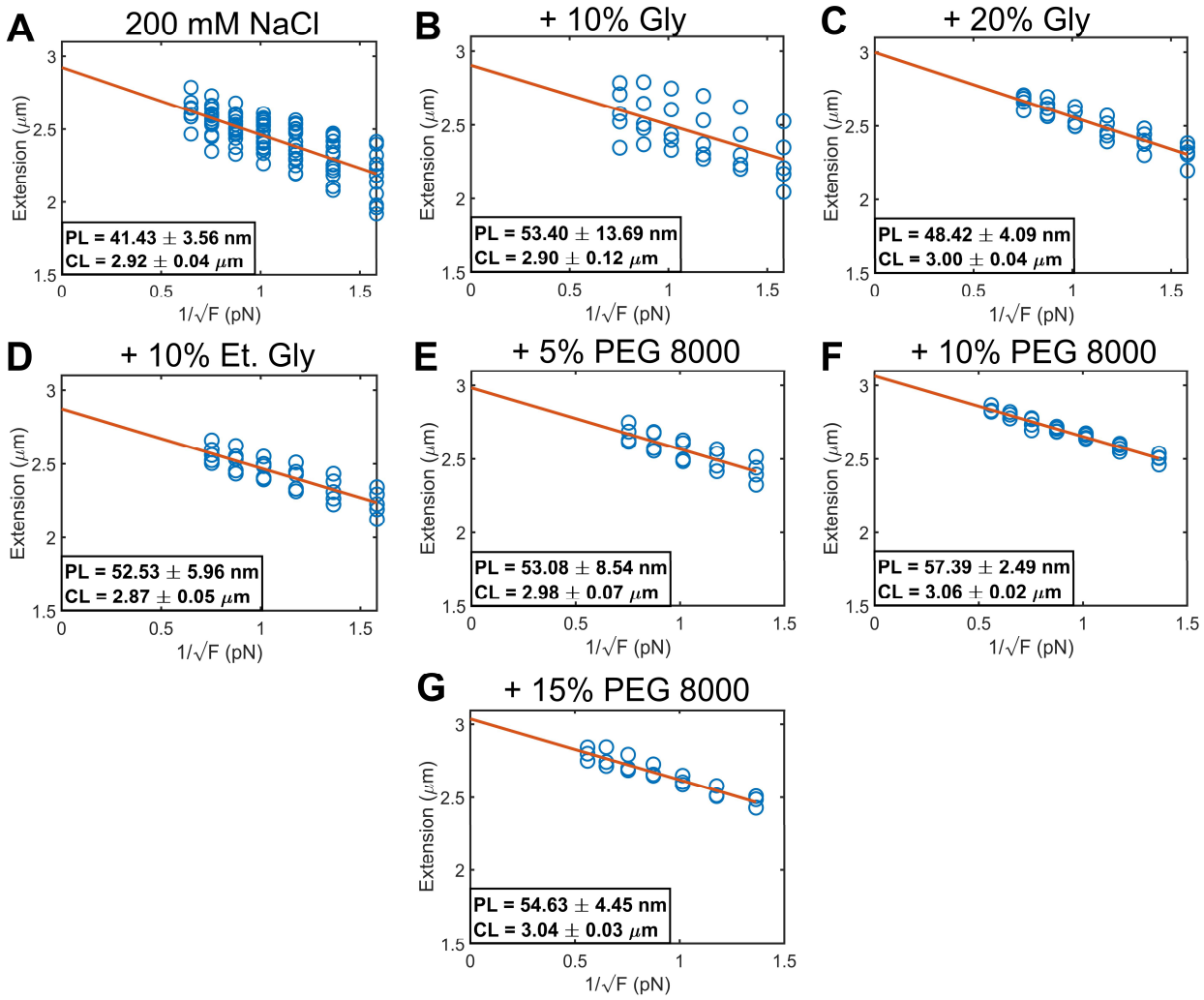


Figure S1. Persistence length (PL) and contour length (CL) of dsDNA in presence of different co-solutes. Persistence length was measured using the slope of the Extension- inverse of the square root of force curve(1). Extension (blue circles) was measure with measured at forces between ~0.8 pN and ~1.5 pN. Slope and intercept of the best linear fit line (red solid line) in plots of the DNA extension versus the inverse of the square root of force were used to determine the persistence length (PL), contour length (CL) and standard errors in accord with expectations from the worm-like chain polymer model.

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