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The dependence of DNA supercoiling on solution electrostatics

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A R T I C L E I N F O

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A B S T R A C T

We develop an elastic–isotropic rod model for twisted DNA in the plectonemic regime. We account for DNA elasticity, electrostatic interactions and entropic effects due to thermal fluctuations. We apply our model to single-molecule experiments on a DNA molecule attached to a substrate at one end, while subjected to a tensile force and twisted by a given number of turns at the other end. The free energy of the DNA molecule is minimized subject to the imposed end rotations. We compute values of the torsional stress, radius, helical angle and key features of the rotation–extension curves. We also include in our model the end loop energetic contributions and obtain estimates for the jumps in the external torque and extension of the DNA molecule seen in experiments. We find that, while the general trends seen in experiments are captured simply by rod mechanics, the details can be accounted for only with the proper choice of electrostatic and entropic interactions. We perform calculations with different ionic concentrations and show that our model yields excellent fits to mechanical data from a large number of experiments. Our methods also allow us to consider scenarios where we have multiple plectonemes or a series of loops forming in the DNA instead of plectonemes. For a given choice of electrostatic and entropic interactions, we find there is a range of forces in which the two regimes can coexist due to thermal motion.

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1. Introduction

The mechanical and electrostatic properties of DNA directly affect various cellular processes, such as replication, transcription, compaction and protein–DNA binding. This is the motivation behind this study of DNA supercoils, which are also known as plectonemes. Plectonemes in DNA molecules are manipulated by several molecular machines during key processes, such as transcription and DNA repair [1]. In several scenarios, the action of these molecular machines or enzymes on DNA has been found to depend on the mechanical stress present in the molecules [2,3]. Consequently, DNA supercoiling remains a subject of study for theorists and experimentalists alike.

Experimentally, DNA supercoiling has been investigated using several biochemical and biophysical methods, including single-molecule experimental techniques, where individual DNA molecules can be stretched and twisted under physiologically relevant conditions [4–8]. In these experiments, it is possible to apply a force and/or moment parallel to the filament axis of a DNA molecule, and to measure the elastic response in terms of elongation and angle of twisting about the filament axis. In rotation–extension experiments, the vertical extension of the DNA filament and the external moment are recorded as a function of the number of turns. It is a well-known feature of the experimental curves that there is a regime, corresponding to the formation of plectonemes, where there is almost a linear relationship between the DNA extension and the applied number of turns. Also, as shown in te recent experiments of Forth et al. [4], Lipfert et al. [5] and Mosconi et al. [6], the external moment is approximately constant in the plectonemic regime.

Plectonemes have been studied theoretically as elastic rods by many authors [9–14]. In order to interpret single-molecule experiments, Purohit [15,16] accounts for the effects of thermal fluctuations as well as electrostatics in plectonemes and straight portions of DNA, and shows that many features seen in the recent experiments of Forth et al. [4] can be qualitatively reproduced using an elastic rod model. Furthermore, as seen in Fig. 5 in Purohit [16], his theoretical results for the slope of the linear region in vertical extension of the DNA vs. number of turns of the bead are around twice the value of those found in experiments by Forth et al. [4]. One of the goals of this paper is to address this problem and get more quantitative agreement with single-molecule experiments. Our approach follows those of van der Heijden et al. [14] and Clauvelin et al. [17,18], who use a variational formulation to solve for the geometry of the plectoneme. The analysis in van der Heijden et al. [14] considers only the elastic energy of the filament, but Clauvelin et al. [17,18] and other authors [19] consider electrostatic interactions together with the elasticity, and are able to reproduce some of the features of the rotation–extension experiments. In agreement

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with Purohit [16], Clauvelin et al. [18] reach the conclusion that electrostatics plays a minor role compared to the elasticity of the DNA in these experiments. Contrary to this conclusion, recent single-molecule experiments and molecular simulations have shown that the results of the rotation–extension experiments depend strongly on the salt concentration of the solution [20,21]. For this reason, we carefully consider electrostatics in this paper and present an analytical model that captures the behavior of DNA in rotation–extension experiments and simulations for a variety of DNA lengths, applied loads and salt concentrations. We also apply our model to a novel set of DNA experiments with a mixture of monovalent and multivalent salts, and show that we can predict the results of these experiments.

Other key variables that are affected by the salt concentration are the discontinuities in extension and torque during the supercoiling transition [20]. These discontinuities have been studied recently by Forth et al. [4] and Daniels et al. [22]. Purohit's models [15,16] capture these discontinuities or jumps qualitatively, but he does not comment on the salt dependence of the jumps. We use our model to provide estimates for the number of turns at which the DNA makes a transition from a straight to a supercoiled configuration, and for the jump in the extension and moment as a function of DNA length and salt concentration. Furthermore, we contemplate the possibility of the formation of multiple plectonemes and other forms of DNA compaction (loops and plectonemes coexistence) due to energetic reasons.

2. General description of the model

We proceed with a model of the plectonemic region of the DNA molecule based on the framework of Clauvelin et al. [18], but we account for thermal fluctuation effects, confinement entropy and an end loop model. The DNA in the experiments is modeled as a Kirchhoff inextensible elastic rod of length $2l$ (with $-l \leqslant s \leqslant l$, where s is the arc length along the centerline of the rod). The Kirchhoff theory of rods models the centerline as a curve in space $\mathbf{r}(s)$ endowed with mechanical properties which are assumed to be suitable averages over the cross-section of the rod [23,24]. The configuration of an inextensible, unshereable rod is defined by $\mathbf{r}(s)$ and an associated right-handed orthonormal director frame $\mathbf{d}_i(s)$, $i = 1,2,3$. For convenience, the vector $\mathbf{d}_3 = \mathbf{r}'(s)$ is taken to be tangential to the rod. The kinematics of the frame are encapsulated in the director frame equations $\mathbf{d}_i' = \mathbf{u} \times \mathbf{d}_i$, where the components of $\mathbf{u} = u_i\mathbf{d}_i$ are measures of the strain, u_3 describes the physical twist, and u_1 and u_2 are associated with bending such that the square of curvature is given by $\kappa^2 = u_1^2 + u_2^2$. We assume a linear constitutive relation between the stresses and the strains, so that the moment $\mathbf{m} = K_b u_1 \mathbf{d}_1 + K_b u_2 \mathbf{d}_2 + K_t u_3 \mathbf{d}_3$, where K_b is the bending modulus and K_t is the twisting modulus. The rod is made up of three regions (see Fig. 1):

- In the linear regions the tails are, on average, aligned with the vertical axis. The tails are not completely straight and the centerline follows a writhing path due to thermal fluctuations in the DNA molecule. An analysis of fluctuating polymers subjected to tension and twist in the straight regime has been carried out in detail by Moroz and Nelson [25,26], where expressions for the twist and writhe have been provided. In our model we will use their expressions.

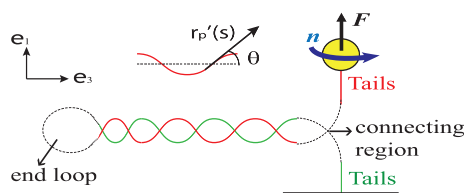

Fig. 1. Sketch representing single-molecule experiments, where a DNA molecule is fixed at one end while the other end is subjected to a pulling force F and twisted by a given number of turns n.

- In the plectonemic region the position vector $\mathbf{r}_p(s)$ and the tangent vector $\mathbf{r}_p'(s)$ describe the superhelix. Note that each helix is itself a piece of double-stranded DNA molecule. So, in the literature, DNA plectonemic geometrical variables (angle and radius) are often referred to as supercoiling or superhelical, to distinguish them from the intrinsic helical nature of the base pair structure. Due to the symmetry of the problem, it is convenient to introduce cylindrical coordinates (r, ψ, z) for the position vector:

$$\mathbf{r}_p(s) = \chi r \mathbf{e}_r + z\mathbf{e}_3 \tag{1}$$

where $\mathbf{e}_3$ is the axis of the helix that wraps around the cylinder and $\mathbf{e}_r = \cos\psi\mathbf{e}_1 + \sin\psi\mathbf{e}_2$. The tangent to the position vector is:

$$\mathbf{r}_p'(s) = \sin\theta\mathbf{e}_\psi + \cos\theta\mathbf{e}_3 \tag{2}$$

$$\psi' = \chi\frac{\sin\theta}{r}, \; z' = \cos\theta, \; 0 < \theta < \frac{\pi}{2}$$

where the chiriality $\chi = \pm 1$ stands for the handedness of the helix: $\chi = 1$ for a right-handed helix and $\chi = -1$ for a left-handed one [17]. The other filament of the plectoneme is obtained by a rotation of π about the helical axis $\mathbf{e}_3$. The plectonemic region is characterized by the helical radius r and the helical angle θ, which are assumed to be independent of the arc length s. The complement $\pi/2 - \theta$ of the helical angle is often referred to as the pitch angle. Both r and θ may depend on the loading. Geometric impenetrability of the helices implies that $\theta \leqslant \pi/4$ [27,28]. Note that the external moment M_{ext} applied in the upper tail of the DNA molecule is equivalent to a total moment M_3 about $\mathbf{r}_p'$ at the beginning of the plectonemic region. By the arguments of conservation of torque about the body axis of an isotropic rod, $\mathbf{m} \cdot \mathbf{d}_3 = K_t u_3 = M_3$ is a constant [24], implying that the twist u_3 is constant in the helical region.[1] One consequence of the use of the expressions given by Moroz and Nelson [26] is that the twist u_3 in the tails is different from that in the plectoneme even though the twisting moment $M_{ext} = M_3$ is the same, since the effective twist modulus is different in each region.

- At the end of the plectonemic region there is a loop. This end loop is formed in the transition from the straight configuration to the plectonemic configuration. In order to model the loop, we propose an approximation based on the localizing solution of an elastic rod [29,30], ignoring thermal fluctuations [31]. For details we refer the reader to Section S.1 of the supplementary data.

The molecule contour length spent per tail is denoted by l_t ($L_t = 2l_t$), the contour length in the loop is denoted by L_o and the contour length per helix is denoted by l_p ($L_p = 2l_p$). The sum of the length of all regions is given by $L = L_p + L_t + L_o$. The equilibrium configuration of the rod is fully specified by the centerline, through the variables r, θ and M_3. In what follows, we compute these parameters as a function of the loading (the pulling force,

[1] At the transition point going from an initially straight state to a plectonemic state there is a jump in the external torque. We define $M_{ext} = M_{critical}$ as the twisting moment in the straight configuration right before the transition (no plectonemes formed), while $M_{ext} = M_3$ is defined as the twisting moment when plectonemes (helices) are present and $\delta M = M_{critical} - M_3$ as the jump in the twisting moment at the transition (see Section 3.1). We use the notation M_{ext} in Section 2 for the external torque. When plectonemes are present, the equations describing the DNA tails can be used by replacing M_{ext} with M_3. When there are no plectonemes in the straight state right before the transition, the equations describing the DNA tails can be used to describe the entire molecule by replacing M_{ext} with $M_{critical}$.

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