Understanding the mechanical response of double-stranded DNA and RNA under constant stretching forces using all-atom molecular dynamics

Alberto Marin-Gonzalez, J. G. Vilhena, Ruben Perez, and Fernando Moreno-Herrero.

Multiple biological processes involve the stretching of nucleic acids (NAs). Stretching forces induce local changes in the molecule structure, inhibiting or promoting the binding of proteins, which ultimately affects their functionality. Understanding how a force induces changes in the structure of NAs at the atomic level is a challenge. Here, we use all-atom, microsecond-long molecular dynamics to simulate the structure of dsDNA and dsRNA subjected to stretching forces up to 20 pN. We determine all of the elastic constants of dsDNA and dsRNA and provide an explanation for three striking differences in the mechanical response of these two molecules: the threefold softer stretching resistance is linked to its more open structure, whereas the opposite twist-stretch coupling of both molecules is due to the very different evolution of its nontrivial force dependence. The lower dsRNA stretching resistance cannot reduce its interstrand distance and can only elongate by unwinding. Interstrand distance is directly correlated with the slide base-pair parameter and its different behavior in dsDNA and dsRNA traced down to changes in the sugar pucker angle of these NAs.

nucleic acid mechanical properties | twist-stretch coupling | DNA | RNA | molecular dynamics

In the past decades, particular effort has been devoted to unveil the complex mechanisms that drive the mechanical response of nucleic acids (NAs) (1–6). They occur in nature in two chemically different molecules, DNA and RNA, with both possessing the ability to form complementary double helices. In vivo, NAs are not typically found in their relaxed forms. Instead, in the biological processes in which they take part, proteins wrap (7), bend (8), stretch (9), and twist (10) double-stranded DNA (dsDNA) and double-stranded RNA (dsRNA) molecules (11). As a result of these mechanical stresses, NA structure and functionality are affected (12). Direct evidence of the interplay between mechanical stress and biological activity has been provided via single-molecule experiments (13, 14). Indeed, techniques such as optical and magnetic tweezers (1, 13, 14) have emerged as essential tools in the characterization of the mechanical properties of dsDNA (1) and, most recently, dsRNA (5, 6, 15).

Single-molecule experiments are performed on very long NA chains (thousands of base pairs), thus making it extremely challenging to access how the mechanical stress changes the NA structure locally (i.e., how it changes at the nanometer scale at which proteins operate). This limitation is especially important for the small forces (below 20 pN) at which most biological processes occur (16). The analysis of single-molecule force extension data with a continuum mechanics approach, the so-called elastic rod model (17, 18), reveals two particularly striking results: the threefold softer stretching response of dsRNA compared with dsDNA (6) and the opposite sign of the twist-stretch coupling (2, 3, 5, 19). Ad hoc model extensions, such as the addition of an outer helical wire (2), and several models at the base-pair level (5, 20) have been proposed to rationalize some of these results. However, no satisfactory explanation for the twist-stretch coupling difference between dsDNA and dsRNA at the continuum or coarse grain level has been found. A satisfactory explanation may involve differences in the atomic-scale structure that are beyond a continuum mechanics analysis (19, 21).

To investigate local NA structural changes induced by mechanical stress, one may resort to all-atom molecular dynamics (MD) simulations. MD can access the atomic detail of an NA structure (22–25) and provide information about the mechanical response of the chain as a whole (19, 23, 26–29). One common approach to address this problem is to determine the elastic parameters of a given NA chain through thermal fluctuations at the equilibrium of an unrestrained sequence (19, 30, 31). However, this approach cannot explore extensions corresponding to large force regimes. This limitation results in the incapability of understanding why dsDNA twist-stretch coupling changes with an applied force (2, 3, 32, 33). An alternative approach computes potential(s) of mean force (PMF) (26, 29, 34). Nevertheless, obtaining a PMF where a given reaction coordinate (e.g., elongation, twist) is sampled enough to ensure thermal equilibrium is reached at each point is a very demanding computational task. As a result, in most MD simulations reported so far, the PMF is computed from many short simulations (t ~ 100 ns). Although they provide very useful insights about conformational changes arising from deformation, the statistical fluctuations of the
observables are too large to provide an accurate quantitative prediction of NA mechanical parameters (29, 35).

Here, we overcome these limitations and provide a comprehensive characterization of the mechanical response of both dsDNA and dsRNA based on microsecond-long, all-atom MD simulations of these molecules under constant stretching forces in the range of 1–20 pN. From the response of the average elongation, the average twist, and the coupling of their fluctuations to the applied force, we determine all of the elastic constants of dsDNA and dsRNA without any prior assumption. Our data agree with general elastic values reported in the literature and reproduce the twist-stretch coupling force dependence measured in single-molecule experiments. A thorough analysis of our simulations provides a firm basis to explain the differences between both molecules. First, we develop the springiness hypothesis into a discrete model to understand the threefold softer stretching response of dsDNA. Second, we explain the opposite sign in the twist-stretch coupling of dsDNA and dsRNA by changes in their interstrand distance. Analysis of our data at the atomic level allowed us to trace down this different behavior and the complex force dependence of the twist-stretch coupling to the response of the slide base-pair parameter, and its relation to the sugar pucker angle.

Results and Discussion

**Determination of All NA Elastic Parameters from MD Simulations.**

We performed all-atom MD simulations using the 16-mer dsDNA and dsRNA molecules used by Liebl et al. (19). Molecules were neutralized with 30 sodium counter-ions and placed in a cubic box with dimensions of ~80 Å × 80 Å × 80 Å, which was then filled with explicit water molecules. Both NA molecules were stabilized at a pressure of 1 atm and a temperature of 300 K, and then subjected to a constant stretching force applied to the center of mass of the atoms belonging to the next to last base pairs of the sequences (Fig. L4). The constant force protocol introduced here is given in SI Appendix. One microsecond-long MD simulations were performed for force = 1, 5, 10, 15, and 20 pN. Molecules remained close to the starting A and B forms during the simulation time at all forces (Fig. 1B and SI Appendix, Figs. S1 and S2). The MD trajectories were then analyzed with AmberTools and 3DNA software (36, 37).

Our simulations were first analyzed with the elastic rod model (17, 18). Because bending fluctuations can be neglected on the length scale of the molecule (19) (SI Appendix), the energy of a stretched and twisted NA molecule subjected to a constant force may be written as:

\[ E = \frac{1}{2} S \frac{x^2}{L} + \frac{1}{2} C \frac{\theta^2}{L} + \frac{g}{L} x\theta - xF, \]

where \( E \) is energy, \( F \) is force, \( L \) is the equilibrium extension at zero force, \( x \) is the elongation or deviation from \( L \), and \( \theta \) is the change in helical twist from its unperturbed equilibrium value. The three constants \( S \), \( C \), and \( g \) are the stretch modulus, the twist modulus, and the twist-stretch coupling constant, respectively. From Eq. 1, one can derive the elastic rod model constitutive equations for an NA molecule (2, 17):

\[ \frac{x}{L} = \frac{1}{S} F, \]

\[ \frac{\theta}{L} = \frac{g}{CS} F, \]

\[ \frac{\Delta x}{\Delta \theta} = \frac{g}{S} \]

where \( S = -S^2/\pi \) is the effective stretch modulus and values are obtained at equilibrium. To determine the NA elastic parameters \( S \), \( C \), and \( g \), it suffices to measure the force dependence of three observables [i.e., the average elongation \( x = f(F) \), the average twist deformation \( \theta = f(F) \), and how thermal fluctuations of the twist are coupled to fluctuations of the elongation \( \Delta x/\Delta \theta \)] (a detailed description is provided in SI Appendix).

As predicted by the elastic rod model, \( x \) and \( \theta \) changed linearly with the applied force (Fig. 2A and B), thus corroborating that the elongation and twist deformation are elastic in the range of forces used here. The slope of a linear fit to the extension data provided the twist unrestrained stretch modulus \( S \) of the dsDNA and dsRNA molecules (Fig. 2A). For both molecules, \( S \) was positive and the values provided by our simulations, \( S_{\text{DNA}} = 1,120 \pm 50 \) pN and \( S_{\text{RNA}} = 416 \pm 7 \) pN, were consistent with experimental results (Table 1) and reproduced the threefold softer behavior of dsRNA previously measured experimentally (6).

The torsional mechanical response of dsDNA and dsRNA (Fig. 2B) showed that as the force increases, DNA overwinds and RNA unwinds. Although counterintuitive, this behavior is in agreement with previous experimental and theoretical works (2, 3, 5, 19). The slope of a linear fit to the helical twist data provided a measurement of \(-g/CS\) (Fig. 2B). Given that both \( S \) and \( C \) are positive, a negative slope leads to a positive value of \( g \) (i.e., unwinding of dsRNA) and a positive slope leads to a negative value of \( g \) (i.e., overwinding of dsDNA). The dsDNA and dsRNA showed twist rigidities of \( C_{\text{DNA}} = 303 \pm 23 \) pN·nm⁻² and \( C_{\text{RNA}} = 310 \pm 24 \) pN·nm⁻², which is in quantitative agreement with experimental measurements (Table 1).

Mining each of our 10 different microsecond MD trajectories following the methods reported by Liebl et al. (19), we were able to determine how the twist-stretch coupling changes with force (Fig. 2C and SI Appendix, Fig. S3). Our data lead to two remarkable observations. First, \( g \) has a negative value for dsDNA and a positive value for dsRNA, providing independent confirmation of the opposite twist-stretch coupling of these molecules. Although these values compared reasonably with a previous theoretical work (19) (SI Appendix), they showed an overestimation in absolute values compared with experimental measurements (Table 1) as discussed further below. Second, the twist-stretch coupling for dsDNA and dsRNA changed with
In the following, we will take advantage of the atomistic detail of our simulations to dwell on the origin of the threefold softer stretching response of dsRNA compared with dsDNA, the opposite sign in their twist-stretch coupling, and its dependence with force.

**Explaining the Striking Difference Between dsDNA and dsRNA Stretch Modulus.** We developed a discrete model based on the springiness hypothesis proposed by Lipfert et al. (5) and Chou et al. (20). The springiness model considers a chain of segments that join the centers of all consecutive base pairs (Fig. 3A). If bending is neglected, the extension \( h \) of the molecule may be written as:
\[
h = h(l, \cos \beta) = l \cos \beta,
\]
where \( l \) is the length of the chain and \( \beta \) represents the angle between the segments of the chain and the helical axis of the molecule, thus providing a measurement of the springiness of the system (Fig. 3B). Here, we propose that an increase in extension can be achieved by either decreasing \( \beta \) or increasing \( l \) (SI Appendix). Therefore, the elongation \( \Delta l \) is, at first order approximation, the sum of these two contributions:
\[
\Delta l = \Delta l_\beta + \Delta l_\gamma.
\]
We calculated \( \Delta l_\beta \) and \( \Delta l_\gamma \) contributions for dsDNA and dsRNA for different forces and obtained a linear behavior (Fig. 3C). This linear response means that the change in extension can be modeled as two springs in a series, with elastic constants \( k_\beta \) and \( k_\gamma \). The inverse of the slopes of linear fits to the datasets \( \Delta l_{\mathrm{dsDNA}}(F) \), \( \Delta l_{\mathrm{dsRNA}}(F) \), and \( \Delta l_{\mathrm{dsRNA}}(F) \) are, respectively, the elastic constants \( S \), \( k_\beta \), and \( k_\gamma \) (Fig. 3C).

Note that \( S \) can also be computed from \( k_\beta \), and \( k_\gamma \), as
\[
S = \frac{k_\beta k_\gamma}{k_\beta + k_\gamma}.
\]
The obtained values for \( S_{\mathrm{dsDNA}} = 1.076 \pm 68 \) pN and \( S_{\mathrm{dsRNA}} = 421 \pm 15 \) pN from the springiness model were in good agreement with the ones calculated from Fig. 2A and Eq. 2, revealing that this simple model captures to a high precision the elastic stretching response of the NAs in this range of forces.

In a system of two springs in a series, the softer spring dominates the global elastic response. Our data show that when an NA is stretched under forces below 20 pN, the major contribution to the change in extension is the springy term, \( \Delta l_\beta \). In other words, the lengthening induced from separating the centers of the base pairs is much smaller than the contribution coming from deforming the chain. Although this result may be expected for dsRNA based on its more open structure, we show here that this claim also holds for dsDNA. This finding contrasts with the intuitive idea that dsDNA stretching should involve base-pair separation due to its nearly straight structure. In fact, a careful inspection of Fig. 3C shows that the contribution of \( \Delta l_\beta \) to the extension is negligible up to 10 pN. Consequently, the qualitatively different stretching elastic response can be attributed to

**Table 1. Elastic constants determined from microsecond-long MD simulations of dsDNA and dsRNA and the constitutive equations of the elastic rod model**

<table>
<thead>
<tr>
<th>Elastic parameter</th>
<th>dsDNA (this work)</th>
<th>dsDNA (exp. work)</th>
<th>dsDNA refs.</th>
<th>dsRNA (this work)</th>
<th>dsRNA (exp. work)</th>
<th>dsRNA refs.</th>
</tr>
</thead>
<tbody>
<tr>
<td>( S ), pN</td>
<td>1,280 ± 70</td>
<td>1,450 to 1,750</td>
<td>(32, 33)</td>
<td>480 ± 11</td>
<td></td>
<td></td>
</tr>
<tr>
<td>( \tilde{S} ), pN</td>
<td>1,120 ± 50</td>
<td>649 to 1,401</td>
<td>(5, 6, 42–44)</td>
<td>416 ± 7</td>
<td>350,500</td>
<td>(5, 6)</td>
</tr>
<tr>
<td>( C ), pN·nm(^3)</td>
<td>303 ± 23</td>
<td>386 to 448</td>
<td>(5, 45–47)</td>
<td>310 ± 24</td>
<td>409</td>
<td>(5)</td>
</tr>
<tr>
<td>( g/kT ), no units</td>
<td>−54 ± 3</td>
<td>−17 to −39</td>
<td>(2, 5, 32, 33, 41, 46)</td>
<td>34 ± 1</td>
<td>11.5</td>
<td>(5)</td>
</tr>
</tbody>
</table>

The constitutive equations of the elastic rod model are shown in Eqs. 2–4. These equations allow us to compute all elastic parameters from the slopes of Fig. 2A and B and from the helical rise-helical twist slopes computed for each force (SI Appendix, Fig. S3). Quoted errors represent deviations from the linear fits. Constants obtained in this work are compared with other experimental (exp.) work.
relative extension, with denoted by either reducing the angle defined by these two parameters. The extension can increase by the next base-pair center, characterized by the three parameters runs around the helical axis of the molecules. This chain deviates from the purple beads represent consecutive base-pair centers and form a chain that molecules. The model is based on the springiness hypothesis (5, 20), where croscopic details of the system. Our constant-force, microsecond-long experiments have reported an opposite twist-coupling sign. Experiments have reported an opposite twist-coupling sign for dsDNA and dsRNA (5), resulting in overwinding of dsDNA and unwinding of dsRNA when the molecules are stretched. If we model the molecules as two strings wrapped around each other, our intuition tells us that they will unwind when stretched (Fig. 4A, Upper). This result supports the idea that overwinding or unwinding is coupled to the change in interstrand separation as suggested above. Alternative methods to calculate the interstrand distance (SI Appendix, Fig. S6) confirmed this result.

Fig. 3. Discrete model explains the different stretching response of dsDNA and dsRNA. (A) Top and side views of dsDNA (Left) and dsRNA (Right) molecules. The model is based on the springiness hypothesis (5, 20), where purple beads represent consecutive base-pair centers and form a chain that runs around the helical axis of the molecules. This chain deviates from the helical axis significantly more for dsRNA than for dsDNA. (B) Each segment is characterized by the three parameters h, l, and β, where l is the distance to the next base-pair center, h is the projection of l on the helical axis, and β is the angle defined by these two parameters. The extension can increase by either reducing β (i.e., increasing cosβ) and/or increasing l. These values are denoted by x_{hl} and x_{l}, respectively. At first-order approximation, the total change in extension can be written as Δh = x_{hl} + x_{l} (SI Appendix). (C) x_{hl}/l and x_{l}/l contributions to the total relative change in extension x/l (same data as in Fig. 2A) for dsDNA (Upper) and dsRNA (Lower). A linear fit constrained to pass through the origin point (1,0) was carried out for each dataset. From the slopes, we calculated k_{DNA} = 1,330 ± 50 pN, k_{RNA} = 522 ± 3 pN, k_{dsDNA} = 5,600 ± 1,500 pN, and k_{dsRNA} = 2,170 ± 140 pN. Error bars in C were calculated as described in Materials and Methods.

Fig. 4. Physical mechanism of the opposite sign of dsDNA and dsRNA twist-stretch coupling. (A, Upper) Double helical structure can overwind when stretched if the interstrand distance is allowed to shrink. (A, Lower) Alternatively, a fixed interstrand distance duplex will unwind when stretched. (B) Relative change of the interstrand distance (Upper) and slide (Lower) with respect to the F = 1 pN value, plotted against the relative increase in the extension induced by force (dsDNA, blue; dsRNA, red). Datasets were fitted to a linear function constrained to pass through the (0,0) point, excluding the value at F = 20 pN for dsDNA (main text). Error bars were calculated as described in Materials and Methods. (C) Cartoon illustrating the relationship between slide and interstrand distance upon stretching. A reduction of slide is accompanied by a reduction of the interstrand distance as it occurs with dsDNA (SI Appendix, Fig. S9B). (D) Representation of two base-pair steps to highlight the different orientation of the sugar with respect to the phosphate backbone of dsDNA (Left) and dsRNA (Right). (E) Fluctuations in sugar pucker angle with respect to the slide parameter. The bin size is 0.02 Å. Data points are mean values with SEM.
of the time for both molecules (SI Appendix, Fig. S8A). For the sake of clarity, we consider a decrease of slide as a decrease of its absolute value, which corresponds to the approaching of consecutive base pairs. Importantly, the interstrand distance and the slide are strongly correlated (i.e., a decrease of slide leads to a decrease of interstrand distance) (Fig. 4C and SI Appendix, Fig. S8). For dsDNA, the slide decreased when stretched, whereas for dsRNA, it barely changed (Fig. 4B, Lower and SI Appendix, Fig. S9). Consequently, dsDNA overwinding (i.e., interstrand distance reduction) can be explained in terms of the reduction of its slide parameter as it elongates (Fig. 4C). Note, however, that both the interstrand separation and slide of dsDNA deviated from the linear behavior at F = 20 pN (discussed in next section). On the contrary, dsRNA was not able to reduce its interstrand distance/slide; therefore, it can only elongate by unwinding.

The different behavior of the slide parameter of dsDNA and dsRNA can be traced down to the most fundamental difference between these two molecules. The connection of the nitrogenous bases to the phosphate backbone is done through a deoxyribose sugar in dsDNA and a ribose sugar in dsRNA. The additional oxygen atom of the ribose sugar results in a distinct orientation with respect to the backbone (40) (Fig. 4D). Moreover, application of force through the phosphate backbone is likely to affect the particular stereochemistry of the sugar. We measured the sugar pucker angle in our simulations and found a homogeneous population of C3'-endo (∼18°) for dsDNA and a more disperse population with an average value of ∼122° for dsRNA. From the fluctuations of the molecules, we observed that although the sugar pucker angle of dsDNA significantly changed with the slide, it remained constant around the C3'-endo configuration in dsRNA (Fig. 4E). This larger flexibility of the sugar pucker angle in dsDNA provides an additional degree of freedom to decrease the slide, allowing overwinding upon stretching. In the dsRNA case, the lack of this degree of freedom precludes reduction of the interstrand separation, resulting in unwinding.

A recent work has attributed the opposite twist-stretch coupling of dsDNA and dsRNA to an opposite change in the inclination parameter when the duplexes are overwound, arguing that the molecules elongate by decreasing this parameter (19). Although this hypothesis provides a very reasonable explanation of the opposite couplings in unrestrained simulations, we have observed that at ∼5 pN, inclination saturates for dsDNA; thus, the molecule no longer elongates by this mechanism (SI Appendix, Fig. S10). The mechanism proposed here, based on the slide, is substantially different from the mechanism proposed by Liebl et al. (19) and allows us to trace from this counterintuitive behavior to the most fundamental difference between dsDNA and dsRNA (i.e., the extra hydroxyl group in dsRNA sugar).

**dsDNA and dsRNA Twist-Stretch Coupling Dependence with Force.** Finally, to understand the dependence of g with the force, we analyzed the fluctuations of the system. The values of the helical rise at each constant force simulation were discretized in bins of 0.02 Å, and the mean helical twist and slide were computed for each bin (Fig. 5 A and B and SI Appendix, Fig. S11). This representation revealed that the coupling between twisting and stretching defines a more complex scenario than a simple straight line, with regions where the molecule overwinds and others where it unwinds. It also emphasized the tight relation between the slide and the helical twist.

In the case of dsDNA, there were two clearly defined regions (Fig. 5A). For helical rise ≤3.3 Å, the molecule overwound when stretched (g < 0), in accordance with the negative sign previously found for g (Fig. 2). For helical rise ≥3.3 Å (green region in Fig. 5A), DNA was no longer able to overwind (helical twist remained approximately constant) and the slope in this region was close to zero (dashed line in Fig. 5A, Upper). As a consequence, molecule excursions into this region lead to a reduction of the average value of g (continuous line in Fig. 5A). A gradual effect can also be observed for intermediate forces (SI Appendix, Fig. S12). As the force increased, the green region became more and more populated (Fig. 5C), such that, at F = 20 pN, its population is large enough to induce a decrease in −g/S of 10% (Fig. 2). The observed flattening in mean slide at 20 pN (Fig. 4B, Lower) can also now be explained by the contribution of data points in the green region (Fig. 5A, Lower). The same argument applies to the fact that the interstrand distance barely changed at 20 pN (Fig. 4B, Upper). At higher forces, we predict an inversion in the trend of the slide, inducing an eventual decrease in the interstrand separation and an inversion of the sign of g. Furthermore, additional simulations at 30 pN confirmed the trend of decreasing −g/S observed at 20 pN. These results are in reasonable agreement with reported experimental measurements (SI Appendix, Fig. S13). This work provides a possible atomic explanation for this experimentally observed effect (2, 3, 32, 33).

Similar reasoning can be applied to dsRNA (Fig. 5B). In this case, when the molecule explored the region with values of helical rise ≤2.4 Å (green region in Fig. 5B), it overwound when stretched (g < 0, dashed line in Fig. 5B, Upper), contrary to the average behavior of dsRNA. Data in the green region contributed to lower the average value of g. As the force increases, this region gets...
Values of the elastic parameters S and C obtained from our MD simulations were in good agreement with experimental data (Table 1). Regarding g, values were in reasonable agreement with recent MD simulations performed at zero force (19), but systematically larger in absolute terms compared with the available continuum analysis providing an explanation of the striking differences found in single-molecule experiments. Furthermore, we showed how a hierarchical analysis from a continuum approach, through a discrete model, to an all-atom description paves the way to link the disparate behavior to structural differences in the arrangement of consecutive base pairs, and to the different sliding between base pairs upon stretching. Our work highlights MD simulations as a powerful tool to unveil the connection between forces and structure of NAs and, possibly, to gain insight into the associated changes in their biological functionality.

Materials and Methods
Simulation, data processing, and constant force protocol details are provided in SI Appendix. In brief, NA duplexes were built using NAB software (36). Energy minimization was carried out in 5,000 steps with restraints on the molecules, followed by an additional 5,000 steps of unrestrained energy minimization. The systems were heated up to 300 K and equilibrated for 20 ns in the NPT ensemble (constant number of particles, pressure, and temperature). Constant force simulations were then run for 1 μs. The first 200 ns were taken as a constant-force equilibration, and all measurements were performed during the last 800 ns. Following the method of Liebl et al. (19), only the 10 central base pairs of RNA were considered in the analysis. Data analysis was carried out using Ambertools software. Errors were computed by splitting the data into five time windows of 160 ns and calculating the SEM considering the measurements in each window.

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