Magnetic tweezers measurements of the nanomechanical stability of DNA against denaturation at various conditions of pH and ionic strength

Alessia Tempesta1, Valeria Cassina1, Doriano Brogioli1, Roberto Ziano1, Simona Erba1, Roberto Giovannoni2, Maria G. Cerrito2, Domenico Salerno1,* and Francesco Mantegazza1

1Dipartimento di Scienze della Salute, Università di Milano-Bicocca, via Cadore 48, Monza (MB) 20900, Italy
2Dipartimento di Chirurgia e Medicina Interdisciplinare, Università di Milano-Bicocca, via Cadore 48, Monza (MB) 20900, Italy

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ABSTRACT

The opening of DNA double strands is extremely relevant to several biological functions, such as replication and transcription or binding of specific proteins. Such opening phenomenon is particularly sensitive to the aqueous solvent conditions in which the DNA molecule is dispersed, as it can be observed by considering the classical dependence of DNA melting temperature on pH and salt concentration. In the present work, we report a single-molecule study of the stability of DNA against denaturation when subjected to changes in solvent. We investigated the appearance of DNA instability under specific external applied force and imposed twist values, which was revealed by an increase in the temporal fluctuations in the DNA extension. These fluctuations occur in the presence of a continuous interval of equilibrium states, ranging from a plectonemic state to a state characterized by denaturation bubbles. In particular, we observe the fluctuations only around a characteristic force value. Moreover, this characteristic force is demonstrated to be notably sensitive to variations in the pH and ionic strength. Finally, an extension of a theoretical model of plectoneme formation is used to estimate the average denaturation energy, which is found to be linearly correlated to the melting temperature of the DNA double strands.

INTRODUCTION

DNA denaturation is at the origin of many biological phenomena, for example, replication, transcription and the interaction of specific proteins with single-stranded DNA. In the present work, we conducted a direct quantitative characterization of DNA denaturation at the single-molecule level. Given the influence of environmental conditions on the biological activity of DNA, we performed measurements in different conditions of pH and ionic strength.

Single-molecule techniques are particularly relevant in the current panorama of biophysical research because they have opened new roads to the analysis of several biological and biomedical problems that are not easily observable with ensemble measurements (1–3). Typical examples of single molecule techniques are magnetic tweezers (MT) (4–8), optical tweezers (9–12), atomic force microscopy (13–16) and single-molecule fluorescence spectroscopy (17,18). Compared with the other techniques, MT methods have a straightforward implementation, and given their capability for applying torsional constrain, MT allows the study of single molecules in conditions that are not easily accessible with the other methods (19,20). Recently, valuable torsional experiments have also been performed with optical tweezers with unconventional nanoparticles (21–23) or by exploiting refined analysis (20,24), but MT manipulation still offers a simpler strategy for applying force and twist to single molecules.

The MT technique is based on the manipulation of a single DNA molecule linked to magnetic spherical...
The capillary flow cell (1 mm² section, 5 cm long, VitroCom, Mountain Lakes, NJ, USA) was prepared according to a method in the literature (41). Briefly, the internal walls were uniformly coated with polystyrene (average molecular weight = 230,000, Aldrich, Milan, Italy) (42) and were coated with 5 µg of sheep polyclonal anti-digoxigenin antibody (Roche, Milan, Italy). The treated surface was passivated for 2 h at 37°C with a solution consisting of PBS pH 7.4, 0.1% of Tween-20, 10 mg/ml of bovine serum albumin (Roche) and 3 mM of NaN₃ (4).

DNA and magnetic bead preparation
The DNA molecule required to perform an MT experiment must be bound at one end to an immobile support (the glass capillary) and at the other end to a magnetic bead (5). The DNA molecule used in this work was 7000 bp long and was assembled by a central part extracted from pCMV6-Neo plasmid (5780 bp, ~1.9 µm) and by two functionalized ends. One end was modified by digoxigenin, and the other end was modified with biotin to provide a connection to the immobile support (the anti-digoxigenin-coated capillary) and to the mobile support (the avidin-coated magnetic bead), respectively. Following the methods previously reported in the literature (6), the DNA tails were functionalized by polymerase chain reaction; thus, the DNA was attached via multiple bonds to the supports. The molecule results torsionally constrained so that the rotation of the magnets induces a real torque on the DNA, which could not swivel around the bonds. As it was already reported (6), we used 1-µm diameter, streptavidin-coated, superparamagnetic beads (Dynabeads MyOne Streptavidin C1, Dynal, Invitrogen, Milan, Italy). We added 1 µl of the bead solution to 10 µl of the functionalized DNA solution for a final concentration of 1 x 10⁶ molecules of DNA per microliters. After 5 min of incubation, the DNA + microbeads solution was diluted in 500 µl of PBS pH 7.4, supplemented with 0.1% of Tween-20 and 3 mM of NaN₃ (referred to hereafter as PBS–Tween-20); the solution was subsequently injected into the capillary. Finally, after 1 h of incubation, we washed out all of the unattached beads with a solution of PBS–Tween-20 pH 7.4.

**PH and ionic strength characterization**
To study the influence of environmental conditions, DNA properties were analysed in solutions having different pH and ionic strength values from those of the standard PBS at pH 7.4. The PBS solutions for measurement in different pH conditions were prepared by balancing Na₂HPO₄ and KH₂PO₄ to obtain different values of pH and by changing the NaCl concentration to maintain constant ionic strength. The resulting values of pH were checked by a standard pH meter (Mettler Toledo, Milan, Italy). The range of pH explored was 5 < pH < 9.

The PBS solutions for measurement in different ionic strength conditions were prepared by modifying only the NaCl concentration (5, 50, 150, 395 and 500 mM) and maintaining the pH at 7.4. The resulting conductivity of the solution was checked by a conductimetre. For measurements at different conditions, at least 10 volumes of the solution was injected into the capillary to ensure complete substitution of the buffer.

**MT technique**
Our MT apparatus followed the most classical proposed schemes (5–7), and it has been previously described elsewhere (41,43,44). The capillary, which is linked to a buffer-flow system, is placed over a homemade inverted microscope, which is composed of an objective (Nikon 100×, 1.25-NA oil immersion with a 15 cm focal-length tube lens leading to an actual magnification of 75×) and is equipped with a piezoelectric focusing system (PIFoc, Physik Instrumente, Bresso, Italy). Images were acquired at a rate of 60 frames per second with a Charge-Coupled Device (CCD) camera (Marlin, Allied Vision Technologies) and werefed into a Personal Computer.
Data analysis

By considering the diffraction images generated at different heights of the bead, illuminated by a light emitting diode (LED) light (45,46), the DNA extension was calculated. A force is exerted (by the magnetic field of the external magnets) on the bead and consequently also on the DNA; the force was measured from the $x$–$y$ fluctuations of the bead, as previously described (7,8). The mean square displacement $\sigma^2 = \langle (x - \langle x \rangle)^2 \rangle$ was evaluated with an appropriate correction for the camera integration time (8,41,47). Next, we extracted the force $F$ with the equipartition theorem (4,7): $F = k_B \cdot T \cdot L_e/\sigma^2$, where $k_B$ is the Boltzmann constant, $T$ is the temperature and $L_e$ is the DNA extension. Once the applied force was calibrated in this manner, we were able to apply a known force to the bead. In the explored regime, the DNA extension $L_e$ is an increasing function of the applied force $F$ and the specific dependence of $L_e$ versus $F$ can be interpreted in the framework of the so-called worm-like chain model (48). By simply rotating the external magnets at a fixed height (and thus at a fixed applied force), we can apply a twist to the torsionally constrained DNA molecule.

RESULTS

We have measured the average extension $\langle L_e \rangle$ of a DNA molecule as a function of the number of imposed turns $n_t$ or analogously as a function of the degree of supercoiling $\sigma = \frac{\Delta L_e}{L_{50}}$ (where $L_{50}$ is the natural linking number in absence of external constraints, and $\Delta L_e$ is the excess or deficit of $L_e$). The obtained data are shown in Figure 1 (upper panel) for the classical representative case of DNA at standard conditions ($\text{pH} = 7.4$, ionic strength $I_s = 150 \text{mM}$) and for two different values of the applied pulling force ($F = 1.2$ and $0.45 \text{ pN}$). The mean value $\langle L_e \rangle$ was obtained by averaging over a time interval of several seconds, as detailed later. The circular labels A, B, C and D located on the data of Figure 1 (upper panel) correspond to the sketches of Figure 1A–D (lower panels), which provide an idea of the actual behaviour of DNA under the actions of torsion and force. As previously described in literature (6) and visually depicted by the sketches of Figure 1, the effects of applied force and imposed twist are as follows: forming a plectoneme (Figure 1C), extending the DNA (Figure 1A and B) and forming a denaturation bubble (Figure 1D). There are detailed experimental studies of the initial stages of plectoneme formation and rigorous nanomechanical models of the buckling and post-buckling behaviour of chains under torsion (49–53). However, we believe that our naïve sketches are still valuable and describe the primary characteristics of the experimental findings.

The $\langle L_e \rangle$ as a function of $n_t$ data are presented in Figure 2 for certain values of the applied pulling force ($F = 1.2$ pN, $F = 0.8$ pN and $F = 0.5$ pN). The results correspond to those of the classical MT literature (6). In this study, we concentrated our attention on the temporal statistical fluctuations of the data and particularly on the standard deviation $\sigma_{L_e}$ of the DNA extension measurements. The histograms in the insets of Figure 2 show the distribution of $\Delta L_e = (L_e - \langle L_e \rangle)$ for the data highlighted by the coloured circles. The histograms are fitted by Gaussian distributions, and the resulting values of the standard deviations $\sigma_{L_e}$ for $F = 0.8$ pN and $n_t < -20$ (i.e. in the transition region between the plectonemic and denaturation states) seem to be larger with respect to other domains of $F$ and $n_t$.

To explore this phenomenon quantitatively, we also calculated the temporal autocorrelation function $g(t) = (L_e(t) L_e(t+\tau))/\langle L_e(t)^2 \rangle$ for various values of the time delay $\tau$. Two examples of the obtained correlation functions and the corresponding original data traces of the DNA extension versus time are presented in Figure 3, panel A and B, respectively. As apparent from Figure 3, the correlation function is negligible for $F = 0.5$ pN, that is, the correlation between the data occurs on a short time interval compared with our acquisition time.
The degree of supercoiling \( \sigma \) (upper x axis) at the various forces \( F \) indicated by the labels. The lateral histograms represent the statistical distribution of \( \Delta L_e = L_e - \langle L_e \rangle \) (in \( \mu \text{m} \)) of the data highlighted by the coloured circles. Data obtained in standard conditions.

For \( F = 0.8 \) pN, the correlation function is a decreasing function over an appreciable duration time of the order of 100 ms. We fitted the correlation functions with an exponentially decaying expression of the form \( g(t) = e^{\frac{-t}{\tau_{\text{char}}}} \), where \( \tau_{\text{char}} \) (the characteristic correlation time) is the free fitting parameter.

The data of the standard deviations obtained as a function of the number of imposed turns \( n_t \) and the applied forces \( F \) are presented in Figures 4A and 5A, respectively. Analogously, the measured \( \tau_{\text{char}} \) values, as resulting from the previously described fitting procedure, are shown in Figures 4B and 5B for different values of the \( n_t \) and \( F \). In Figure 4A and B, we have also showed the error bars as coloured bands around the data. Given the acquisition frame rate of the camera, we choose a value of 50 ms as a confidence threshold. The data below that threshold are not reliable. To emphasize this consideration, we have added to the figures showing \( \tau_{\text{char}} \) a dashed horizontal line, indicating the limit of validity of the experimental fitting procedure for extracting \( \tau_{\text{char}} \).

It is worthwhile to note that to avoid systematic statistical errors, the values of \( \sigma_{Le} \) and \( \langle L_e \rangle \) are obtained by making a statistical average, that is, collecting data during a time interval of \( \sim 100 \) of the corresponding \( \tau_{\text{char}} \). However, we have verified that the plotted values of \( \sigma_{Le} \), \( \tau_{\text{char}} \) and \( \langle L_e \rangle \) were not modified by increasing further the time interval of the statistical averaging. As clearly shown by Figures 4 and 5, the data seem to be more noisy and more correlated in a specific range of \( n_t \) and \( F \). Indeed, the values of the standard deviation \( \sigma_{Le} \) and \( \tau_{\text{char}} \) below a fixed negative value of \( n_t \) (\( n_t < -20 \)) are larger with respect to other domains of \( F \) and \( n_t \). The values of \( \sigma_{Le} \) and \( \tau_{\text{char}} \) show both a clear maximum around a characteristic value of the force (\( F_{\text{char}} = 0.8 \) pN). The value of \( F_{\text{char}} \) coincides for both \( \sigma_{Le} \) and \( \tau_{\text{char}} \). The obtained \( F_{\text{char}} \) seems to not depend on \( n_t \) within the experimental errors, and the maximum values of \( \sigma_{Le} \) and \( \tau_{\text{char}} \) at \( F = F_{\text{char}} \), in the range explored, are decreasing functions of \( n_t \).

The domains with large values of \( \sigma_{Le} \) and \( \tau_{\text{char}} \) are located in regions where, under the action of the imposed turns and forces, the DNA is fluctuating between the denatured and plectonemic states, continuously exploring all the intermediate states. Because the possibility for DNA denaturation is strongly dependent...
on the acid–base or ionic characteristic of the solvent in which the DNA is dispersed (54), we have repeated the aforementioned analysis for different values of pH and $I_s$.

To investigate this point, we measured $\langle L_v \rangle$ versus $n_i$ at fixed force for various values of $\mu$ and $I_s$, and the results are presented in Figure 6A and B, respectively. In this study, we directed our attention to the data at negative $n_i$, where a mild or flat dependence of $\langle L_v \rangle$ as a function of $|n_i|$ is a clear indication of the induced mechanical denaturation of the DNA molecule. As noticeably demonstrated in Figure 6A, the denaturation occurred more easily for pH values far from pH 7.4, where the hydrogen bonds of the bases are weaker (55). Similarly shown in Figure 6B, the DNA is easily denatured for negative $n_i$ at $I_s = 5$ mM, that is, at low ionic strength, where the screening of the electrostatic repulsion of the two double strands is reduced.

More quantitatively, in Figure 7A–D, we have plotted the extracted values of the standard deviations and the characteristic correlation times measured as a function of the applied $F$ for different pH and $I_s$ at negative imposed turn $n_i = -40$. From the entire ensemble of data, we extracted the values of the characteristic force in the different situations. The data show that at pH values other than pH 7.4 or for low ionic strength, the DNA transition to denaturation is shifted to lower values of $F_{\text{char}}$, that is, the DNA is less stable. By considering Figure 7, we can observe that the distribution of forces over which the DNA length fluctuations occur is systematically larger for high ionic strength (and less systematically for non-physiological pH). Indeed, the denaturation occurs at lower forces in acid or basic environments or at low ionic strength, as shown in Figure 8A and B, where the $F_{\text{char}}$ values are plotted as functions of pH and $I_s$.

The observed bell-like shape of $F_{\text{char}}$ versus pH and the increasing dependence of $F_{\text{char}}$ versus $I_s$ are notably similar to the classical dependences observed for the DNA melting temperature $T_m$ as functions of pH and $I_s$. Indeed, in Figure 8C and D, for comparison we plotted the behaviour of $T_m$ versus pH and $I_s$ as classically reported in literature (56,57). The similarity between the

Figure 4. Standard deviation $\sigma_{L_s}$ of the DNA extension (Panel A) and characteristic time $\tau_{\text{char}}$ of the correlation functions of the DNA extension fluctuations (Panel B) measured as a function of the number of imposed turns $n_i$ (lower x axis) and of the degree of supercoiling $\sigma$ (upper x axis). The horizontal dashed line represents the reliability threshold of the fitting procedure for obtaining $\tau_{\text{char}}$, because of the finite acquisition time of the experiments. The coloured bands represent the error bars (confidence regions) as obtained by considering the quality of the fitting procedure. Data obtained in standard conditions at the different forces $F$ indicated by the labels.

Figure 5. Standard deviation $\sigma_{L_s}$ of the DNA extension (Panel A) and characteristic time $\tau_{\text{char}}$ of the correlation functions of the DNA extension fluctuations (Panel B) measured as a function of the force $F$. In all cases, the estimated errors are below the 20% of the measured values. The horizontal dashed line in Panel B represents the reliability threshold of the fitting procedure for obtaining $\tau_{\text{char}}$, because of the finite acquisition time of the experiments. Data obtained in standard conditions at the different number of imposed turns $n_i$ indicated by the labels. The vertical line indicates the characteristic force $F_{\text{char}}$. 
behaviours confirms the capability of the method in characterizing the stability of DNA in different situations.

DISCUSSION

In the experimental results presented in this report, we mainly described the behaviour of DNA for negative imposed turns and for characteristic values of the applied force, where the DNA extension fluctuations are largely enhanced; the enhanced fluctuations are observed because molecules can choose between the mechanical options of locally denaturing (bubbles) or forming supercoils (plectonemes). We observed that such fluctuations are strongly dependent on the ionic strength and on the pH of the solvent.

We now recall the main results of a simplified quantitative model introduced in (40) which can describe all of the experimental findings. According to that model (40), when the DNA is twisted, the phenomenon of denaturation or plectoneme formation is accompanied by the stored energy $E_{tot} = E_{twist} + E_{plect} + E_{den} = \frac{B}{2}\left(2\pi n_p\right)^2 + \frac{B}{2R_pL_0} + F + \alpha n_n$. Where the first term is the twisting energy ($E_{twist}$), the second is the energy accumulated during plectoneme formation ($E_{plect}$) and the third one is the denaturing energy ($E_{den}$). $L_0$ is the extended length of the DNA, $n_p = (n_t - n_d - n_{nt})$ is the number of turns that store energy in the twist, $n_d$ is the number of loops of radius $R_p$ along the DNA, $n_n$ is the number of turns relaxed by a partial DNA denaturation, $B$ is the DNA bending constant, $C$ is the DNA torsion constant and the constant $\alpha$ is proportional to the binding energy between the bases of the two DNA single strands. The first two terms were already used in model (6), whereas the last one was originally introduced in (40).

The plectonemic energy has a double origin: the work necessary for bending a $2\pi R_p$ portion of the double strand and the work for pulling the bead in presence of the force $F$. Disregarding the denaturation contribution, by minimizing the energy $E = E_{twist} + E_{plect}$, one can obtain the standard results of the equilibrium plectoneme radius $R_p = \sqrt{\frac{B}{2}F}$ and the buckling number $n_b = L_0\sqrt{\frac{BF^2}{\pi^2}}$, as previously reported in the literature (6).

The situation is more complicated when the denaturation contribution energy $E_{den}$ is explicitly taken into account. The boundary conditions prescribe for $E_{tot}$ a triangular validity region in the plane $(n_p,n_d)$ with $n_t < n_d < 0$, $n_t < n_d < 0$ and $n_p + n_d < n_t$. The model (40) predicts the presence of a characteristic force $F_{char}$, where the energy necessary for relaxing a new turn by forming a loop or by denaturing the double strands is equal. The model analytically relates this characteristic force to the bending constant $B$ and to the parameter $\alpha$: $F_{char} = \frac{\alpha}{B} n_t$.

The representative energy landscapes for three different values of the external force $F$ are plotted in Figure 9A ($F < F_{char}$), Figure 9B ($F = F_{char}$) and Figure 9C ($F > F_{char}$) as a function of $n_p$ and $n_d$. The darker regions in Figure 9A, B and C represent the equilibrium points or lines of minimum energy. The energy landscapes are obtained assuming standard values of $B = 50 \cdot k_B \cdot T$, $C = 140 \cdot k_B \cdot T$, $L_0 = 1.88 \mu m$ and $\alpha = 1.12 \cdot 10^{-19}$ J. In the first case, when $F < F_{char}$, the energy landscape presents a minimum for $n_p = n_t - n_d$ (i.e. plectoneme formation) and $n_d = 0$ (i.e. no denaturation). In the case when $F > F_{char}$, the minimum of the energy is attained for $n_d = n_t - L_0 \cdot \alpha/(4C\pi^2)$ (i.e. the force is enough to form denaturation bubbles) and $n_p = 0$ (i.e. no formation of plectonemes). In the intermediate situation, when $F = F_{char}$, the minimum of the energy landscape is represented by a line, suggesting that the force is allowing a fluctuation along the line connecting the two states.

Using the experimental value $F_{char} \approx 0.8$ pN and a DNA persistence length of $\sim 50$ nm, the resulting value of $\alpha$ is $\sim 1.1 \cdot 10^{-19}$ J (766 kJ/mole), which is approximately eight times the classically reported average value of the free energy per DNA base pair, $\Delta G \approx 8.4$ kJ/mole (58–60). It seems then necessary to open eight base pairs to relax one turn. This number of base pairs is consistent with the pitch of the double helix. This value is obtained not considering the influence of other possible structures [e.g. hairpin (61) or cruciform (62)]. The
obtained value of $\alpha$ can be easily compared with the measured denaturation torque reported in (63). The parameter $\alpha$ is obtained by calculating the work necessary for twisting the DNA of one turn: $\alpha = \Gamma \cdot 2\pi$ (where $\Gamma$ is the torque). By considering the reported torque of $11pN \cdot nm$, the calculated value for $\alpha$ is $0.7 \times 10^{-19} J$, consistent with what observed in the present work.

Using the proposed energy expression $E_{tot}$ and a Boltzmann distribution enables a prediction for $h_L$ and $\sigma_L$ in the different situations. The calculated average DNA extension $h_L$ is presented in Figure 9D as a function of $nt$ for three different force values. In this study, we suppose that the DNA extension variations are mainly because of plectoneme formation ($L_c = L_0 - 2\pi R \cdot n_t$). We also assume that the denaturation phenomenon does not introduced a significant DNA length variation given the low $nt$ values at which the experiments are performed. These $nt$ values correspond to a ssDNA of 300–350 bp, which is $<5\%$ of the DNA length. Evaluating the extension length of such ssDNA, and assuming 1 nm of persistence length, the maximum extension variation should be of the order of few 10th of nanometres, which is not relevant in our case. In the present calculations, we also introduced a worm-like chain model dependence of the extended value of the DNA length $L_0$ (64), and we also verified that the force dependence of the constant $C_0$ is not strongly influencing the results (65). Comparing Figures 2 and 9D, we note that the model qualitatively describes not only the classical plectonemic behaviour observed for $n_t > 0$ but also the
transition between the plectonemic and denaturation regions for $n_t < 0$.

The qualitative agreement between the data and the model is also demonstrated by comparing the behaviour of the calculated $\sigma_L$ as a function of $n_t$ shown in Figure 9E and the experimental findings shown in Figure 4A. Both the calculated and measured fluctuations increase for $n_t < 0$ and in a specific range of $F$. The qualitative agreement is confirmed by Figures 9F and 5A, where we have shown the calculated and measured values of $\sigma_L$ as a function of $F$. The similarity of the theoretical predictions and the data in all of the situations confirm the capability of the model to describe the characteristics of the experimental findings.

There are certain obvious quantitative differences between the measurements and the theory. For example, the calculated fluctuations show a transition from the plectonemic state towards the denaturating state in a narrow transition region of the force ($\pm 10\%$ of the $F_{char}$), whereas the experiments report a range larger ($\pm 20\%$ of the $F_{char}$) than the model. This larger experimental fluctuation amplitudes with respect to the theoretical ones are presumably because of the fact that our model neglects several aspects of the real system, such as the presence of an initiation energy for starting the denaturation bubble (52), the possibility to have different bubbles along the DNA, the occurrence of different plectonemic regions (66) and the existence of thermal oscillations of the plectoneme radius (53). These considerations are also presumably the reason why the proposed model does not explain the observed dependence of the distribution of forces, over which the DNA fluctuations occur, on ionic strength or pH (Figure 7). In particular, an influence of the buffer condition on the number of plectoneme domains has been recently demonstrated (67). All these not considered ingredients can be at the origin of the quantitative differences between data and simulations. Nevertheless, the presented results reproduce qualitatively all the experimental conclusions, and the modelling can provide a deeper insight into the characteristics of DNA denaturation under the effects of external constraints. Other DNA structures (Z-DNA or L-DNA) can be formed at $n_t < 0$ (68–70), but all such structures are present in non-physiological conditions, far from the low force and turn regime, which is the object of the present work.

To consider the data taken in non-standard conditions of the solvent, we recall that the classical phenomenon of DNA denaturation that occurs at the temperature $T_m$ depends on the specific DNA base composition and on environmental conditions (54). Indeed, at the temperature $T_m$, the hydrogen bonding between the bases weakens, and the two strands separate. $T_m$ is a strongly dependent function of the pH and the ionic strength, as reported in the classical measurements (57,56) shown in Figure 8C and D.
We assume that the DNA melting occurs when the number of bases in the denaturation bubbles is comparable with half of the number of DNA base pairs. Considering a simplified version of the Poland–Scheraga model as reported in (71), the melting temperature \( T_m \) is related to the energy \( E_{cl} \) for elongating a bubble by one base pair, by the relation \( \frac{E_{cl}}{RT_m} = \alpha \cdot \ln(2) \). Considering \( E_{cl} = \alpha b \), where \( b \) is the number of base pairs opened to relax one loop, we predict a linear relationship between \( \alpha \) and \( T_m \) with a theoretical proportionality constant \( m \) of \( \sim 1.5 \cdot 10^{-22} J/°K \). In Figure 10, the values of \( \alpha \), which are plotted as a function of the melting temperature measured at different pH and \( I_0 \), confirm this linear behaviour with a constant \( m = 3.4 ± 1 \cdot 10^{-22} J/°K \). The order of magnitude agreement validates the appropriateness of the proposed model.

CONCLUSION

The data and the corresponding model shown in the present work elucidate new, innovative and fundamental aspects of DNA biophysics, specifically, the nanomechanical stability of single DNA molecules under various environmental conditions in a force and torsion regime compatible with those involved in important protein activities, such as DNA and RNA polymerases (72, 73) and DNA gyrase (74). The presented research opens the way for further studies and investigations in more sophisticated and realistic situations; these studies could examine modified DNA structures or the effects of the presence of DNA binding proteins, enzymes and ligands.

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