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Conformational Flexibility of DNA

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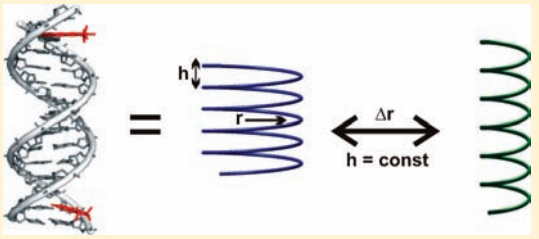
Supporting Information

ABSTRACT: Pulsed Electron−Electron Double Resonance (PELDOR) on double-stranded DNA (ds-DNA) was used to investigate the conformational flexibility of helical DNA. Stretching, twisting, and bending flexibility of ds-DNA was determined by incorporation of two rigid nitroxide spin labels into a series of 20 base pair (bp) DNA duplexes. Orientation-selective PELDOR experiments performed at both X-band (9 GHz/0.3 T) and G-band (180 GHz/6.4 T) with spin label distances in the range of 2−4 nm allowed us to differentiate between different simple models of DNA dynamics existing in the literature. All of our experimental results are in full agreement with a dynamic model for ds-DNA molecules, where stretching of the molecule leads to a slightly reduced radius of the helix induced by a cooperative twist−stretch coupling.



INTRODUCTION

The dynamical behavior of DNA plays an important role in the specificity and energetics of DNA−protein interactions. They are of fundamental importance for the understanding of cellular processes such as replication and transcription, where DNA is bent or twisted upon interaction with specific sites of proteins. Although mechanical and elastic characteristics of double-stranded DNA (ds-DNA) have been studied extensively,[1] recent experiments reported unexpected dynamical behavior, contradicting the classical model of an elastic rod.[2,3] Using rotor bead tracking experiments,[4] a twist−stretch coupling[5] on a single ds-DNA molecule was accessed. In these experiments, a fluorescent rotor bead was attached to a nick in the center of a 7 kbp long ds-DNA molecule, which was stretched by moderate forces of 4−30 pN with a magnetic bead attached to the end of the DNA molecule. The surprising experimental finding, that ds-DNA overwinds under extension, described by a negative twist−stretch coupling,[5] was modeled by a stiff wire representing the sugar phosphate backbone wrapped around the elastic rod representing the helix core. A positive Poisson ratio for the elastic rod leads to a reduced diameter under tension and overwinding of the wire if the helical pitch remains unchanged (Figure 1, Model B). Also, small-angle X-ray scattering interference (SAXSI) studies[6] between two gold nanocrystals attached to both ends of short ds-DNA molecules (up to a length of 35 base pair (bp)) revealed nonclassical behavior. The variance of the end-to-end length $\langle \Delta L^2 \rangle$ of these ds-DNA molecules was found to depend quadratically on the number of bp, contrary to the expected linear dependence for an elastic rod. The data were interpreted to reflect an accordion-like stretch motion of the ds-DNA with a cooperative increase in the pitch over several helical turns (Figure 1, Model A). In contrast to the single molecule experiments, that were performed under tension, a stretch modulus S at least 1 order of magnitude lower was found. To complete the confusion, two alternative explanations of the SAXSI data invoked effects related to the gold cluster rather than DNA itself: repulsive excluded volume interactions between the two gold clusters[7] and a leverage of the DNA fluctuations by the linker construct,[8] which allows us to reproduce the observed data within a standard, noncooperative rigid base-pair model.[9] Leveraging of this type can also be included into cooperative models by accounting for the off-axis position of the gold-cluster makers.[10] In particular, this allows us to test if a cooperative bending mode (Figure 1, Model C) could be responsible for the observed effects. Thus, a conclusive description of the dynamics of ds-DNA molecules under natural conditions is still missing.

Here we show that Pulsed Electron−Electron Double Resonance (PELDOR)[11,12] with a new type of rigidly attached spin label[13,14] can be utilized to disentangle bending, twisting, and stretching motions of short ds-DNA molecules. Since we attach small markers at well-defined positions on the DNA double helix, the excluded volume effects and the uncertainties related to the marker geometry and elasticity of the small-angle X-ray interference (SAXSI) experiments[6,8] should not be an issue for the present study. While a comparison to the noncooperative rigid

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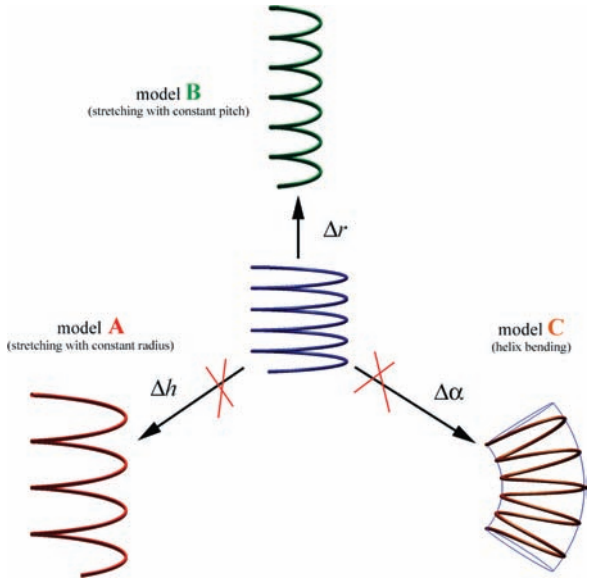

Figure 1. Three possible models for cooperative stretch motion of ds-DNA. Stretching of the DNA is achieved either by variation of the helical pitch of the wire (A) or by variation of the radius of the elastic core (B). In both cases the contour length of the helical stiff wire is kept constant. The effect of radius or pitch change is strongly exaggerated in the graphic to emphasize the differences between the two models. Alternatively, a simple bending model of the DNA structure (Model C) is discussed.

base-pair model[8] is beyond the scope of the present work, we can show that our results are compatible with a cooperative fluctuation model proposed by Mathew-Fenn et al.[6] In particular, our results are in full agreement with a breathing motion of ds-DNA, where the pitch height of the helix is kept constant while the helix radius and molecule length change in a correlated manner (Figure 1, Model B), whereas our data are in contradiction with the other two models (A and C) described above. We observe a ±6% length variance of the 20-mer ds-DNA molecules, reflecting the stretch modulus determined by SAXSI experiments.[6] Moreover, our analysis reveals a negative twist−stretch coupling, corresponding to a radius change of 11% (±65 pm). This value is in full accordance with the twist−stretch coupling parameter of $g = -47$ pN nm derived from single-molecule fluorescence measurements on long ds-DNA molecules.[4] However, bending motions of the short 20-mer ds-DNA molecules in our study are negligible, excluding an explanation of the SAXSI data by Model C (Figure 1). This drastically increased bending rigidity of short ds-DNAs in comparison to long ds-DNAs, and there is a much softer stretching modulus compared to single molecule fluorescence microscopy measurements,[4] which are important new findings. They should be taken into consideration for more elaborated models of DNA dynamics[9,15] as well as for the mechanistic understanding of specific DNA−protein interactions,[16] for example, during DNA repair, in the future.

EXPERIMENTAL SECTION

All PELDOR[11,12] (pulsed electron−electron double resonance) measurements were performed in frozen aqueous buffer solution at a temperature of 40 K, using the dead time free 4-pulse PELDOR sequence,[17] where the refocused echo (caused by three microwave pulses at the probe frequency ν_{probe}) is recorded as a function of the delay time T of the fourth microwave pulse with the pump frequency

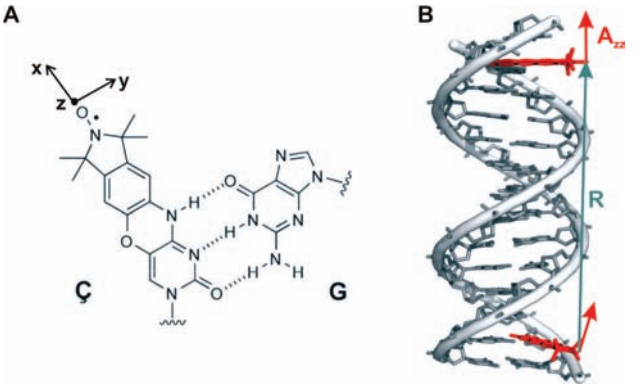

Figure 2. (A) Rigid spin label Ç, a cytidine analogue, base-paired with guanine. The magnetic axis system of the nitroxide hyperfine tensor A and the G-tensor are coplanar to the molecular axis system (x,y,z). The paramagnetic center (unpaired spin) is located at the center of the N−O bond. (B) The duplex DNA(1,12) containing the two spin labels Ç shown in red. The numbers in parentheses indicate labeled base pair positions. For further nomenclature, see the Supporting Information text (section S3). The interconnecting distance vector R and relative orientations of nitroxides can be determined by the PELDOR experiments.

ν_{pump}. Probe and pump frequencies are chosen in such a way to select subensembles of nitroxide molecules with specific orientation with respect to the external magnetic field. For the X-band experiments (~9 GHz/0.3T), the pump frequency was set at the center of the nitroxide spectrum, whereas the probe frequencies were varied. G-band PELDOR experiments (~180 GHz/6.4 T) were carried out with a fixed offset between pump and probe frequency, $\Delta\nu = 70$ MHz, at magnetic field positions corresponding to the resonance conditions for the main canonical orientations of the nitroxide. More information is given in the Supporting Information Text (section S2).

RESULTS AND DISCUSSION

PELDOR spectroscopy allows us to measure the magnetic dipole−dipole interaction between two spin labels covalently attached to the ds-DNA molecule and therefore enables distance determinations in the range of 2−7 nm with high accuracy.[18−21] A rigid nitroxide spin label Ç,[13,14] an analogue of 2′-deoxycytidine (dC) (Figure 2A), was incorporated pairwise into ten 20-mer duplex DNAs. The distance between the labels was systematically varied by keeping one label in a fixed position and walking the other a full turn of a helix. More information is to be found in the Supporting Information text (section S3). These spin labels act as molecular cantilevers and allow monitoring both the distance R between the two unpaired electrons and the distance distribution ΔR of the molecular ensemble. More importantly, the relative orientation between the two nitroxide moieties can be ascertained, which is crucial for distinguishing between bending, torsional, and stretching motion.[21,22]

The ability to determine the mutual orientation of the two nitroxides arises from the anisotropy of the magnetic hyperfine and spin−orbit interactions, which allows selection of nitroxides with a specific orientation with respect to the external magnetic field by the pump and probe pulse frequencies.[23,24] Especially at high magnetic field all orientations of the nitroxide molecule are spectrally resolved. Therefore, parameter-free unique solutions of the relative orientation as well as the distance between the two nitroxides in the nucleic acid can be obtained.[25] The PELDOR

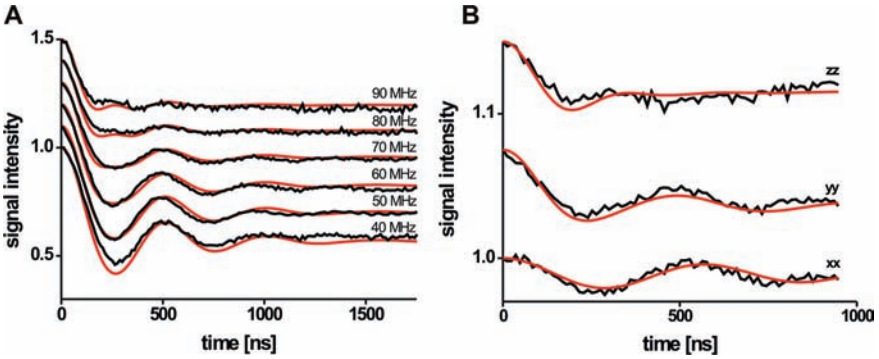

Figure 3. (A) Experimental (black) and simulated (red) X-band PELDOR time traces for different offsets $\Delta\nu$ (in MHz) between probe and pump pulse frequency for DNA(1,9). (B) G-band PELDOR time traces at the three magnetic field positions corresponding to the main G-tensor components. In these experiments, the difference between pump and probe frequency was kept constant at $\Delta\nu$ = 70 MHz.

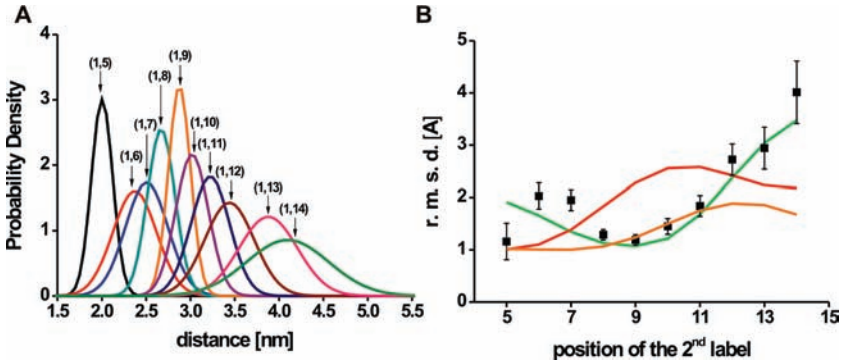

Figure 4. (A) Distance distribution functions based on X-band PELDOR data. Sum of all pump−probe offsets, $\Delta\nu$, data are simulated with a Gaussian distance distribution. (B) rmsd of the distance distribution, $(\langle\Delta R^2\rangle)^{1/2}$, as a function of separating bp. Predictions based on Model B with varied cylinder radius, Model A with varied helix pitch, or Model C based on bending (see Figure 1) are shown in green, red, and orange, respectively. A minimum $(\langle\Delta R^2\rangle)^{1/2}$ results for DNA(1,9).

signal oscillates with the frequency of the magnetic dipole−dipole interaction, which is given by

$$\nu_d = 52.16\frac{1-3\cos^2\theta}{R^3}\,(\mathrm{MHz\cdot nm^3}) \qquad (1)$$

Here, R is the distance between the two unpaired electrons, and θ describes the angle between the distance vector R and the external magnetic field. The overall measured PELDOR time trace consists of a sum of cosine functions of those ds-DNA molecules in the sample where one nitroxide is on-resonance with the probe pulse frequency. Simulation of the PELDOR oscillation frequency as a function of the probe frequency leads to an independent determination of the distance R and the relative orientation between the two nitroxides in a ds-DNA molecule.[21] The damping of the PELDOR oscillation contains the information of the conformational heterogeneity of the DNA duplexes in the frozen state.

For a direct comparison of our experimental results with the predictions from models A−C, we simulated PELDOR time traces based on these models for ds-DNA dynamics, taking the parameters for stretch, twist, and bend motion as described in the literature. Satisfactory agreement with all experimental results (including the complete set of double labeled ds-DNA molecules and all X- and G-band PELDOR time traces) could only be obtained with model B described in Figure 1. This model assumes a variation of the radius of the elastic rod of 0.65 Å by keeping the pitch of the stiff wire constant. Exemplarily, the experimental data for DNA(1,9) overlaid with simulations based on model B are shown in Figure 3. The experimental PELDOR data for all other spin-labeled positions and simulations based on the three dynamic models A, B, and C can be found in the Supporting Information text (section S4). It should be mentioned that only the high-field G-band PELDOR data exclude substantial bending contributions to the dynamic of short ds-DNA molecules and allowed detection of the twisting motion of the molecules.

Our data therefore support the cooperative stretch−twist dynamics (model B) observed on long ds-DNA molecules by fluorescence measurements under tension.[4] The twist−stretch coupling is negative and accounts for an angular variation of ±22° between the N−O axis of nitroxide spin labels positioned one helical turn above one another. This corresponds to a value of g = −47 pN nm, as determined from the single molecule fluorescence measurements.[4] The length variance of ±6% of 20-mer ds-DNA extracted from our experimental data is in full accordance with the SAXSI measurements.[6] As they concluded, this corresponds to a stretch modulus about 1 order of magnitude lower compared to single molecule fluorescence measurements under tension. This surprising finding might be related to the entirely different length of ds-DNAs used for the single molecule measurements, with a high probability of kinks or mismatches.[6]

The determined length variation corresponds to a change in radius of ±65 pm (11%) for model B. Our experimental PELDOR data are neither in agreement with a cooperative helix pitch change (model A) nor with bending of the DNA (model C).[10]

To compare our experimental results directly with the SAXSI data, which were performed on ds-DNA molecules of the same length, we created a new data set by summing the X-band PELDOR time traces for a given ds-DNA molecule over all measured frequency offsets $\Delta\nu$. This procedure efficiently removes the orientation correlation between the two spin labels,[26] allowing determination of the distance distribution function, as reported for the SAXI experiments. The variance $\langle\Delta R^2\rangle$ of our measurement does not increase monotonically with the number of bp's between label positions like the SAXSI data but instead oscillates (Figure 4). This observation is expected from the off-axis positioning of the labels[8,10,14,21,22] and is in agreement with the twist−stretch coupling predicted by model B.

Due to the off-axis positioning of the unpaired electron spins of the two nitroxides relative to the helical axis and their spiral motion arising from the twist−stretch coupling, the distance R between the two unpaired electrons of the two nitroxides is $R = ((R_{long}^2) + (R_{trans}^2))^{1/2}$, where R_{long} is the distance along the axis of the DNA and R_{trans} is the distance in the plane perpendicular to the DNA axis. While the longitudinal distance R_{long} always increases by stretching of the molecule, this is not valid for the transversal distance R_{trans}. Depending on the specific position of the spin label and on the dynamic model considered, this distance might decrease or increase with stretching of the ds-DNA molecule. For DNA(1,9), only Model B predicts a negative interference between ΔR_{trans} and ΔR_{long} and a minimum in the variance $\langle\Delta R^2\rangle$ for 9 bp distance in-between the spin labels for a negative twist−stretch coupling, in full agreement with the experimental observations (Figure 4). Thus, the rigidly attached spin labels allow for a direct observation of this coupling. This was not possible in the SAXSI experiment, where only the distance could be measured. Therefore, these measurements did finally not allow full discrimination between model A, B, or C. On the other hand, the perfect agreement of the PELDOR and SAXSI data (obtained in cryogenic frozen solutions and in liquid solutions at room temperature, respectively) further supports the assumption that the frozen ensemble of conformers determined by the PELDOR method resembles the conformational space at physiological conditions. Similar behavior was found for model compounds,[24] proteins,[27] and RNA[28] by comparison with NMR or MD data.

CONCLUSION

In summary, PELDOR experiments performed at multiple magnetic fields (0.3 and 6.4 T) allowed determination of the conformational flexibility of ds-DNA molecules with minimal perturbations of the native system. This demonstrates the high potential and accuracy of the PELDOR technique for the determination of distances and distance distribution in the nanometer range on macromolecules. Analyzing our experimental data, bending, twisting, and stretching dynamics can be readily distinguished, and thus several simplified models proposed to describe ds-DNA dynamics can be easily tested and compared. These findings should be very helpful in the future for a more sophisticated and elaborate modeling of ds-DNA dynamics, based on stochastic and molecular dynamics modeling,[9,15] which is beyond the scope of this paper. A detailed picture of such dynamics of small ds-DNA molecules will be important to fully understand recognition and transcription processes in cells. Further work on DNA molecules with more complex tertiary structures and investigation of changes in DNA structure and dynamics upon binding to proteins or under different ionic strengths are underway in our laboratory.

ASSOCIATED CONTENT

Supporting Information. Sample preparation, PELDOR experimental parameters, and the comparison of all experimental time traces with calculations based on Models A, B, and C. This material is available free of charge via the Internet at http://pubs.acs.org.

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