COMMUNICATION

Dynamics of nucleic acids at room temperature revealed by pulsed EPR

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Abstract: Investigation of structure and conformational dynamics of biomolecules under physiological conditions is challenging for structural biology. Although pulsed electron paramagnetic resonance (like PELDOR) techniques provide long-range distance and orientation information with high accuracy, such studies are usually performed at cryogenic temperatures. At room temperature (RT) PELDOR studies are seemingly impossible due to short electronic relaxation times and loss of dipolar interactions through rotational averaging. We incorporated the rigid nitroxide spin label Ç into a DNA duplex and immobilized the sample on a solid support to overcome this limitation. This enabled orientation-selective PELDOR measurements at RT. A comparison with data at 50 K revealed averaging of internal dynamics, which occur on the ns time range at RT. Thus, our approach adds a new method to study structural and dynamical processes at physiological temperature in the <10 μs time range with atomistic resolution.

Pulsed electron paramagnetic resonance (EPR) spectroscopy is established as a valued method for investigations of biomolecular structures and conformational flexibility, next to nuclear magnetic resonance (NMR), X-ray crystallography, optical or infrared spectroscopy. Most common are pulsed electron-electron double resonance (PELDOR, also called double electron-electron resonance/DEER)[1,2] or double quantum coherence (DQC)[3] experiments. These techniques rely on the detection of the magnetic dipolar interaction between unpaired electrons. For this purpose, two spin labels are covalently attached to specific sides of the system by side-directed spin labelling (SDSL).[4] Such labelling can be performed either during the synthesis of a biopolymer or post-synthetically.[5,6] PELDOR spectroscopy in combination with SDSL is not limited by the size of the molecules under investigation and can determine distances in the 1.5-10 nm range for nucleic acids, proteins and their complexes.[7–9]

Due to the intrinsically fast echo dephasing time of nitroxide spin labels at higher temperatures, PELDOR experiments are usually carried out in frozen solutions at ~50 K.[10] Thus, such experiments report on a static frozen conformational ensemble, providing the full conformational accessible space of flexible biomolecules at the freezing temperature.[11] However, freezing may trap biomolecules in specific conformations. Furthermore, cryo-protectants, necessary additives to form a good glass upon freezing, can also affect natural equilibria.[12] Additionally, kinetics for dynamic processes are not accessible in frozen solution.

A longstanding goal is to relate PELDOR data acquired at low temperatures to physiological conditions. In order to perform PELDOR measurements at room temperature (RT), one needs to overcome two fundamental limitations which are not present in frozen solutions. First, the rotational motion of the molecules has to be inhibited to avoid averaging out the dipolar interaction. This can be achieved by e.g. immobilization on a solid support. Second, the transverse relaxation time T_2 of the spin label has to be long enough to obtain an appropriate time window for the evolution of the dipolar coupling.

A proof of concept for RT DQC experiments has been demonstrated using triarylmethyl (TAM, trityl) labels attached to a biotin-immobilized protein[13] and on double-stranded DNA.[14,15] As the echo-dephasing mechanism in the liquid phase is strongly driven by the anisotropy of the hyperfine- and g-tensor, the carbon-based trityl radicals with isotropic hyperfine couplings and very small g-tensor anisotropy are good candidates for RT experiments. However, incorporation of the bulky and hydrophobic trityl label into biomolecules is limited, e.g., to end groups or loops and requires an adequate linker to overcome steric hindrance.[16–19] Spirocyclohexyl-derived nitroxides have also been utilized as spin labels on DNAs[15] and proteins[21] to obtain distance information by PELDOR at RT. Here the absence of the rotating methyl groups on the carbon atoms adjacent to the nitroxide functional-groups leads to longer relaxation times. Nevertheless, for both trityls and spirocyclohexyl nitroxides there are indications that the linkers and the attachment strategy still limit the transverse relaxation time T_2.[16] In the case of nitroxides, the molecules had to be immobilized in solid matrices to prevent the tumbling motion of the biomolecule and the nitroxide spin-label. Poly-sugars, like trehalose and sucrose, exhibit great immobilization and cryo-protection properties. However, the degree of dehydration affects the biomolecular structure[19] and full immobilization requires very high sugar concentrations.[22] Thus, the structure and especially the conformational flexibility of the biomolecules under investigation might be affected by this immobilization procedure.

Here we report the use of rigid spin labels for RT PELDOR. The rigid spin label **Ç** ("C-spin")[23] is a cytidine analog where the nitroxide is fused to the base such that Watson-Crick base pairing to guanine is maintained (Fig. 1). The spin label does not perturb

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Supporting information

the helical structure of dsDNA, as demonstrated by X-ray crystallography,[24] and has been shown to be an excellent tool for PELDOR measurements of DNA[25–27] as well as its ribo-derivative **Çm** for RNA[28,29]. Moreover, the rigidity of Ç yields information on the mutual orientation between both spin labels, in addition to the distance. Due to this and its rigid attachment to the biomolecule, the conformational dynamics of nucleic acids can be observed with unpreceded precision.

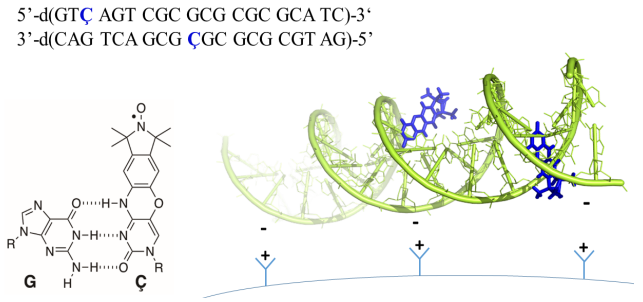

Figure 1: Sequence of the double stranded 20mer DNA sample. The rigid nitroxide spin-label **Ç** base-paired with guanine (**G**) (left). Molecular model of the dsDNA molecule with nitroxide spin labels (blue) and electrostatic interaction to the silica beads (Nucleosil surface/ right).

We synthesized a duplex oligonucleotide containing two **Ç** spin labels with a distance of 2.65 nm for proof-of-principle experiments. This dsDNA had previously been characterized by PELDOR measurements at cryogenic temperature, which revealed the conformational flexibility of the DNA.[23,26,27] The measurements reported on the ensemble of all possible conformations; yet, this is fundamentally a static view. At RT, in contrast, the dynamics of the molecule will modulate the Larmor frequency of the spins within their evolution time and therefore affect the observed PELDOR time trace. Anisotropic couplings which are modulated by the dynamics much faster than their coupling strength will be averaged out.

Synthesis of the spin-labeled dsDNA was performed by phosphoramidite chemistry on controlled pore glass (CPG) as previously published.[30] Subsequently, the spin-labeled dsDNA was adsorbed to functionalized silica-surface beads (Nucleosil) through electrostatic interactions (Fig. 1). Continuous wave (CW)-EPR data proved that the dsDNA was immobilized upon binding to the Nucleosil (Fig. S1). At 50 K, the transverse relaxation time T_2 of the DNA sample was found to be 3 µs for protonated solvent and around 8 µs for deuterated solvent (Fig. S3). The relaxation time in buffered solution at RT was too short to be measured. After immobilization on the Nucleosil surface T_2 was found to be around 550 ns. This time is similar to those obtained with nitroxides immobilized in glassy trehalose.[16] As T_2 depends on the spectral position, the rates are measured on the maximum of the spectrum, represented in Fig. S2. The Boltzmann population difference between the two-electron spin states decreases with increasing temperature, reducing the signal-to-noise ratio of the PELDOR experiment. Fortunately, the longitudinal relaxation rate T_1 also decreases, thus allowing faster signal averaging because of much shorter shot repetition times (SRT).

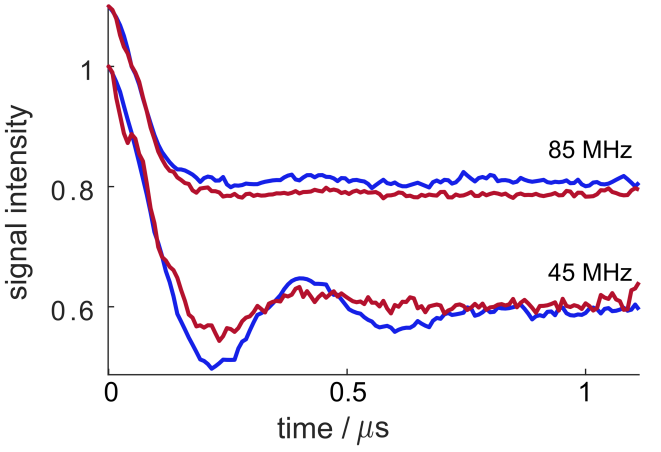

Figure 2. PELDOR time traces at 50 K of dsDNA in a 20% glycerol/water mix (blue) and attached on Nucleosil surface (red). Traces were recorded with 45 MHz (lower trace) and 85 MHz (upper trace) frequency offset between pump and probe pulses. The pump pulse was set resonant to the maximum of the nitroxide spectrum.

X-band PELDOR measurements of the spin labeled dsDNA were performed at 50 K with and without binding to Nucleosil. The two time traces obtained with 45 MHz and 85 MHz offset between pump and probe pulses showed orientation selection[27] but also revealed some differences between each other (Fig. 2). Attaching the dsDNA to the surface increases the dampening of the time traces (Fig. 2 red line, Fig. S4). This is presumably a consequence of the electrostatic interaction between the positively charged Nucleosil surface and the negatively charged backbone (Fig. 1), leading to a somewhat larger conformational distribution of the dsDNA.

Upon increasing the temperature to 295 K, orientation selection remains visible in the PELDOR time traces at both offsets frequencies (Fig. 3A, Fig. S5). Additionally, the RT traces of Nucleosil-immobilized dsDNA samples show a reduced dampening of the dipolar oscillations compared to the 50 K measurements. This goes in line with the assumed averaging of fast conformational dynamics at RT. MD simulations[31] (at RT) of this specific dsDNA revealed bend and twist-stretch dynamics which reflect the conformational ensemble probed by PELDOR at cryogenic temperatures very well. These conformational flexibility is responsible for the dampening of the PELDOR oscillations at low temperature. As these dynamics have a correlation time shorter than 0.5 ns at room temperature, the modulation of the dipolar interaction arising from these dynamics is averaged out (Fig. 3B).

For both frequency offsets, the dynamic averaged ensemble calculated from MD trajectories is in qualitative agreement with the experimental observed PELDOR time traces at RT, whereas the full ensemble of MD structures corresponds well with the low temperature PELDOR experiments, as previously reported[31]. Slight changes of the low temperature PELDOR time traces of the dsDNA sample with Nucleosil reveals structural modifications introduced by the electrostatic interaction. Nevertheless the damping of the oscillations in the PELDOR time traces stays the

same. Therefore, we conclude that binding of the dsDNA to Nucleosil does not inhibit its internal dynamics, leading to the more pronounced dipolar oscillations at room temperature.

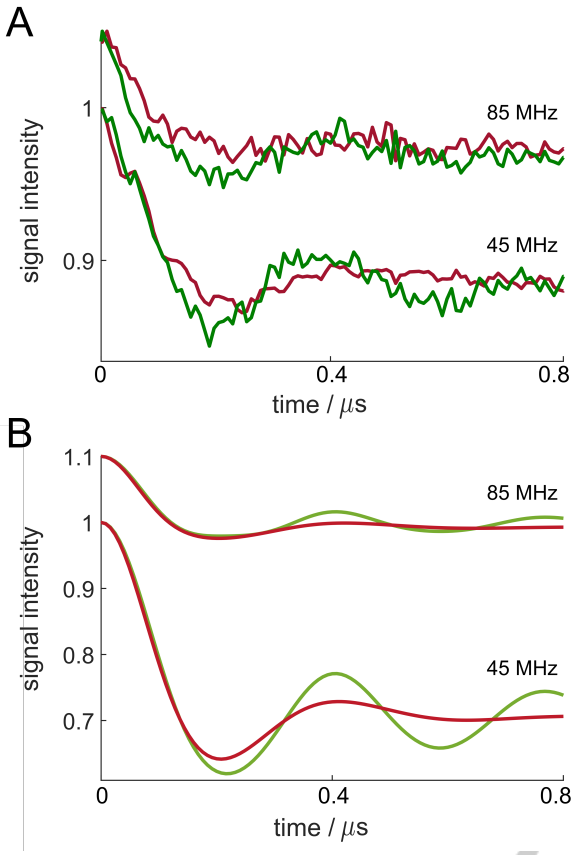

Figure 3. PELDOR time traces of the dsDNA adsorbed on Nucleosil. The traces were recorded with 45 MHz (lower traces) and 85 MHz (upper traces) offset between pump and probe pulse. The probe pulse was set to the maximum of the nitroxide spectrum to gain higher signal to noise (S/N). A) Experimental PELDOR time traces at 50 K (red) and at RT (green). B) Calculated PELDOR time traces from MD trajectories for 50 K (red) and for RT (green). For simulation details see SI.

In conclusion, we have demonstrated that **Ç** is very well suited for PELDOR investigations on DNA molecules performed at physiological temperatures. Since **Ç** is a rigid spin-label, the overall immobilization of the dsDNA is sufficient to obtain a long enough transversal relaxation time T_2 for RT PELDOR measurements. This guarantees minimum distortion of the structure and conformational dynamics of the DNA molecule. The additional orientation information obtained with this rigid spin label enables sensitive detection of even small changes of these properties. Our measurements showed that the attachment to the silica beads (Nucleosil) not only prevents the overall tumbling of the DNA molecule but also changes the conformational distribution slightly. Furthermore, the orientation selective PELDOR data at RT show for the first time the effect of dynamic averaging of fast internal motions. The orientation selective PELDOR time traces are in full agreement with predictions based on MD simulations. Such details are not accessible with flexible spin labels. Thus, our approach offers direct access to structural and dynamical investigations under physiological conditions on such molecules.

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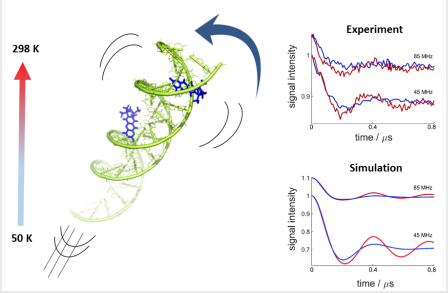
COMMUNICATION

Incorporation of the rigid nitroxide spin label Ç into a DNA duplex and immobilizing the sample on a solid support allowed orientation-selective PELDOR measurements at room temperature. Comparison with the low temperature (50 K) PELDOR data and MD simulations revealed averaging of the fast internal dynamics on the ns time scale at room temperature. Thus, this new approach allows the study of structural and dynamical processes on the low μs time scale at physiological temperature with atomistic resolution.



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