DNA Structures

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Double Electron–Electron Resonance (DEER): A Convenient Method To Probe DNA Conformational Changes**

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Site-directed spin labeling combined with pulsed EPR spectroscopy represents a promising and powerful tool to elucidate the structures of chemical and biological systems, such as DNA, RNA, proteins, and protein complexes.[1,2] Recently, weak interactions in trimeric protein structures, which are usually difficult to detect by other methods, were studied by double electron–electron resonance (DEER) distance measurements.[3,4] Nanometer distances for the above-mentioned biomacromolecules have been determined by DEER[5–7] or double quantum coherence–EPR spectroscopy.[8,9] Whereas conformational changes have previously been detected on protein structures[10,11] and peptides[12] by the DEER technique, here we present the first example of the study of DNA conformational changes by using this method.

Our model system was the B–A transition,[13] which occurs in the formation of the double-stranded regions in RNA, or which is induced by spore proteins or in the presence of organic solvents, such as ethanol and methanol. The DEER technique, because of its several advantages compared to other well-known spectroscopic methods,[13] can quantitatively monitor B–A transitions to elucidate the role of this conformation in several biological processes.[14,15]

Double site-directed spin-labeled (SDSL) DNA structures were made by the selective introduction of a 2,2,6,6-tetramethylpiperidine-*N*-oxyl (TEMPO)-type spin probe on the N2 position of the guanine base (^{TEMPO}**G**). This procedure involves the substitution of an amino derivative of TEMPO on 2-fluorohypoxanthine (^{F}I).[16] As described elsewhere,[5] this derivatization pattern does not significantly alter the overall structure of the duplex. Four DNA duplexes were synthesized, two with both spin probes inserted on the same strand [(4;19) and (4;20)] and two containing one spin probe on each DNA strand [(4;14′) and (4;18′)] (Figure 1a and b). Selected CD spectra for the duplexes without nitroxide spin probes exhibit no differences compared to those registered on the double SDSL duplexes (data not shown).

Buffered aqueous solutions of the SDSL duplexes, which are known to adopt a B conformation, were characterized by DEER. Interspin distances up to 5.6 nm were measured, as summarized in Table 1. The rigidity of the DNA structure was confirmed by a narrow distance distribution obtained by the Tikhonov regularization method.[1] The B–A transition was induced by a high concentration of trifluoroethanol (TFE; higher than 70%, v/v).[13] For all the duplexes studied, a shorter distance was observed in TFE/aqueous buffer relative to the buffered aqueous solution. These distance changes were attributed to an A-like conformation in the presence of TFE. In particular, a remarkable distance change (Δr = 0.8 nm, Figure 2) was observed for the duplex (4;20) in [D_3]TFE/D_2O (80:20). By stepwise addition of TFE we could detect a gradual change of the interspin distances for duplexes (4;14′) and (4;18′).

Five solutions were prepared containing from 0 to 85% (v/v) of TFE (higher concentrations induce DNA precipitation). Figure 3 shows the distance changes for duplex (4;18′). Interestingly, in TFE/H_2O (65:25, v/v) both species can be observed, with two maxima centered at 4.9 and 4.3 nm, which are the distances obtained for the B and A forms, respectively. Another structure that usually adopts an A-like conformation is the (DNA:RNA) hybrid duplex;[14] its conformation is indistinguishable from the A conformation of DNA obtained by CD analysis. On the contrary, the DEER method allowed different distances to be measured for two (4;19) duplexes (see Table 1 and the Supporting Information).

The distances measured for SDSL duplexes exhibit slight deviations from theoretical B/A distance ratios because of the size and placement of the nitroxide spin probes. The distance determinations described herein are supported by molecular dynamics (MD) studies, as confirmed by the values reported in Table 1. Such studies can also shed light on the relative

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Supporting information for this article is available on the WWW under http://www.angewandte.org or from the author.

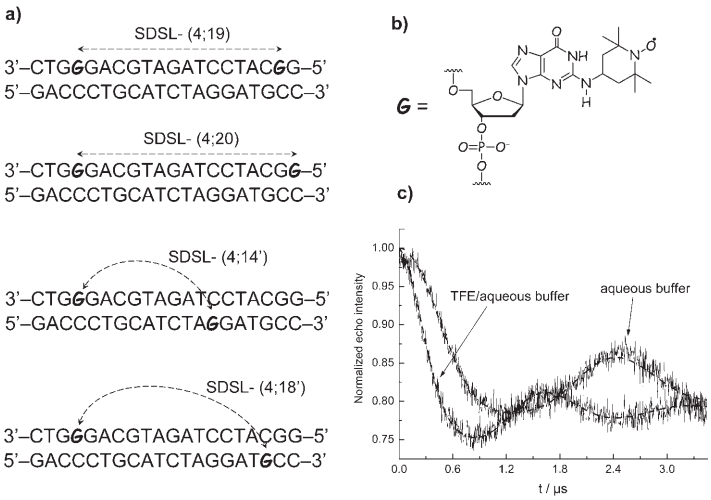

Figure 1. a) SDSL DNA structures studied in this work. b) Derivatized guanine as TEMPO-type spin probe (^{TEMPO}**G**). c) Normalized echo decay for the double SDSL (4;18′) duplex in aqueous buffer (100%, 100 µm) and in TFE/aqueous buffer (85:15, 10 µm). The dashed curves are the echo decay fitted by DeerAnalysis 2006.1.

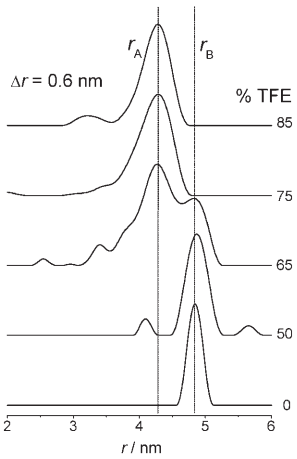

Figure 3. Distance changes as a function of the stepwise addition of TFE to the aqueous solution containing the double SDSL (4;18′) duplex.

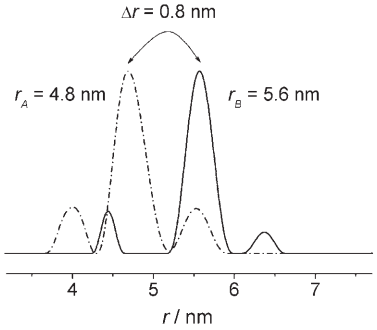

Figure 2. Distance distribution for the double SDSL (4;20) duplex in deuterated aqueous buffer (——) and in deuterated aqueous buffer/[D_3]TFE (20:80, v/v; –•–•).

positions of the nitroxide spin probes, which confirms the A-like forms.

A very good correlation between the distances measured by DEER and the average distances obtained from MD simulations was obtained. The O–O distances extracted from the calculations (on the NO• moiety, Figure 1b) are closer to the spin–spin distances deduced by the DEER technique. In general, the modeled distances are slightly underestimated (ca. 5%) except for the duplex (4;14′), where they are overestimated by about 15%. In the case of the duplex (4;14′), the nitroxide spin position of the second probe (14′) is closer to the center of the duplex compared to the 20, 19, or 18′ positions, and probably induces a more pronounced deformation of the overall structure of the duplex that is not detected in the other systems due to end fraying. Further studies on the placement of ^{TEMPO}**G** bases are currently being carried out.

These promising results, obtained by DEER analysis, represent a significant improvement compared to the well-known continuous-wave EPR[17] and CD[14,15] techniques. Furthermore, among several distance distributions described herein, it was even possible to distinguish between two different A-like conformations. These measurements confirm that DEER analysis is a useful tool to investigate DNA-damage structures and DNA–protein complexes. Conformations obtained by DNA-structure-modulating agents, such as intercalating molecules or nucleic acid analogues (peptide nucleic acids and locked nucleic acids) might also be studied. It would provide new insights into the genotoxicity of selective damage and the DNA repair process by selected proteins, as a result of its precision and the wide range of distances that can be measured.

Table 1: Distance measurements *r* [nm] obtained by DEER and MD studies.

DNA duplex	"B form"			"A form"		
	Experimental distance (DEER)	O–O distance (MD)	N–N distance (MD)	Experimental distance (DEER)	O–O distance (MD)	N–N distance (MD)
(4;20)	5.6 ± 0.2[a]	5.6 ± 0.3	5.5 ± 0.3	4.8 ± 0.2	4.5 ± 0.4	4.5 ± 0.3
(4;19)	5.1 ± 0.2	5.1 ± 0.3	5.1 ± 0.3	4.6 ± 0.3	4.4 ± 0.4	4.3 ± 0.3
(4;18′)	4.9 ± 0.2	4.8 ± 0.4	4.7 ± 0.3	4.3 ± 0.3	4.2 ± 0.4	4.2 ± 0.4
(4;14′)	3.2 ± 0.2	3.6 ± 0.3	3.5 ± 0.2	2.8 ± 0.3	3.3 ± 0.3	3.2 ± 0.3
$(4;19)_{Hybrid(DNA:RNA)}$	n.a.	n.a.	n.a.	4.8 ± 0.3	4.5 ± 0.3	4.5 ± 0.3

[a] The error value is full width at half height of the distance distribution. n.a.: not applicable.

Experimental Section

DEER measurements were performed at 60 or 70 K using a Bruker EleXsys 580 spectrometer equipped with a 5-mm split-ring resonator. Before insertion into the resonator, samples containing 10–15% glycerol as a cryoprotectant were frozen in liquid nitrogen. The four-pulse DEER pulse sequence $(\pi/2)_{\nu 1}-\tau_1-(\pi)_{\nu 1}-\tau-(\pi)_{\nu 2}-\tau_1+\tau_2-\tau-(\pi)_{\nu 1}-\tau_2-$echo was used. Background echo decay was corrected using a homogeneous three-dimensional spin distribution or polynomial baseline correction. The frequency of the signal modulation (Figure 1c) for the dipolar interaction between the spin probes can be related to the interspin distance (through the point-dipole approximation, which can be applied to the nitroxide spin probes) by using the following Equation (1).[1]

$$r = \left[\frac{52.04}{\nu}\right]^{\frac{1}{3}} \quad (1)$$

Herein, r is in nanometers and ν is in megahertz. The distance distribution was calculated by fitting the corrected dipolar evolution data (Figure 1c) using Tikhonov regularization as implemented in the DeerAnalysis 2006.1 package.[18] Further details about the pulse sequence are provided elsewhere.[1,2]

All DNA sequences (as depicted in Figure 1) were prepared by solid-phase DNA synthesis through standard phosphoramidite chemistry. The complementary RNA fragment was obtained by solid-phase assembly using improved Pac-phosphoramidite chemistry.[19] Site-specific insertion of the 2-fluorohypoxanthine residue was performed by using a commercially available phosphoramidite monomer (GlenResearch, Sterling, VA). Then, selective single- or dual-nitroxide DNA labeling was performed as previously described.[16] Finally, DNA fragments containing TEMPO residue were purified by reverse-phase HPLC and analyzed by MALDI-TOF mass spectrometry. DNA duplexes were built by mixing complementary DNA strands in a 1:1 ratio, followed by the addition of an appropriate amount of the hybridization buffer (final concentration: 50 mM KCl, 10 mM Tris-HCl, 1 mM ethylenediaminetetraacetic acid, pH 7.5), heating to 90°C for 3 min, and then slowly cooling to room temperature. The final solutions (60 μL) contained 10% (v/v) glycerol (as cryoprotectant) and 10–100 μM of double SDSL DNA duplexes. Solutions of TFE/H_2O were used for duplexes (4;18′) and (4;14′), whereas deuterated TFE ([D_3]TFE) and D_2O (Fluka) were used for duplexes (4;19) and (4;20). Deuterated matrices were used to increase the transverse relaxation time of electron spins. The B-to-A transition was induced by the stepwise addition of TFE to the buffered aqueous solution of the DNA samples (for duplexes (4;14′) and (4;18′)), up to the H_2O/TFE ratio 15:85.

Modeling: The initial models for the duplexes were built in A-DNA or B-DNA form using the Leap module of the AMBER 9.0 program. The geometry and the charge distribution of the ^{TEMPO}**G** base were computed using the Gaussian 03 program and fitted with the Resp program for compatibility with the force field. After minimization, structures were hydrated with TIP/3P water molecules and Na^+ counterions were added to obtain electroneutral hydrated models. These structures were used to initiate 10 ns of MD production. For the B-DNA and the hybrid DNA–RNA duplexes, no constraints except for the hydrogen bonds were applied. For the A-DNA duplexes, additional constraints on the torsion angle δ (84°) of the sugars and on distances between adjacent phosphate groups (5–6 Å) were applied during the calculations to force the A-DNA geometry. Otherwise an A-DNA-to-B-DNA transition was observed after 500 ps. These simulations were then analyzed to compare the experimental distance measured by DEER and the O–O or N–N average distances of the probe extracted from the MD calculations.

Supporting Information: Distance distributions for the titrations with TFE for the double SDSL (4;14′) duplex, the distance distribution for the double SDSL (4;19) duplexes in the B form and in the A-like forms, and the (4;19) duplex structure derived from the MD study are available.

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