Site-Directed Spin Labeling of DNA Reveals Mismatch-Induced Nanometer Distance Changes between Flanking Nucleotides

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ABSTRACT: Multiple forms of DNA damages such as base modifications, double-strand breaks, and mism pairings are related to inheritable diseases, cancer, and aging. Here, the structural changes of duplex DNA upon incorporation of mismatched base pairs are examined by EPR spectroscopy. Two ethynyl-7-deaza-2'-deoxyadenosine residues separated by two nucleotides were incorporated in DNA and functionalized with 4-azido-2,2,6,6-tetramethyl-piperidine-1-oxyl (4-azido TEMPO) by the click reaction. Mismatches such as dT:dT or dA:dA mispairs were positioned between these two spin labels in DNA duplexes. Pulse EPR experiments reveal that the mismatch-induced local conformational changes are transmitted to the flanking nucleotides and that the impact of this mismatch depends on the nearest neighbor environment.

INTRODUCTION

2'-Deoxyribonucleic acid (DNA) is the carrier of genetic information in living organisms. DNA mutations such as nucleobase changes, double-strand breaks, and mispairings are associated with inheritable diseases, cancer, and aging. In vivo, DNA mismatches are usually caused by the misincorporation of nucleotides during replication or mutagenesis caused by chemical compounds or ionizing radiation. This can lead to conformational changes of DNA domains with biological impact in respect of mutagenicity. Consequently, such alterations, if not repaired, have severe effects on the cellular machinery. Efficient initiation of the cellular mismatch repair system requires the discrimination between intact and damaged DNA sites by the repair machinery. A key component in the cellular repair system is the protein MutS, which has the capability to scan DNA for mismatches thereby testing the shape and flexibility of DNA. The repair efficiency seems to correlate with the induced conformational changes rather than with the thermodynamic stability of the mismatches.

Conformational changes can be monitored by intramolecular distance measurements, which provide new insights into structure–function relationships. Nanometer distance measurements related to mutagenic events involving DNA structural changes have been determined by high-resolution NMR spectroscopy, fluorescence resonance energy transfer (FRET), and X-ray crystallography. Recently, the high sensitivity and accuracy of pulse electron double resonance (PELDOR, or double electron–electron resonance, DEER) on spin labeled oligonucleotides have been demonstrated under conditions close to the physiological state. Several techniques have been developed for the spin labeling of nucleic acids. The highly exergonic character of the copper(1)-catalyzed Huisgen–Sharpless–Meldal alkyne–azide cycloaddition (CuAAC), the so-called click reaction, was used for spin labeling of DNA on solid-phase by Sigurdsson and co-workers and in solution by the Seela and Steinhoff groups. A 7-ethynyl derivative of 7-deaza-2'-deoxyadenosine was incorporated in an oligonucleotide, followed by the click functionalization with 4-azido-2,2,6,6-tetramethyl-piperidine-1-oxyl (4-azido TEMPO) leading to derivative 3 (Figure 1).

This reaction occurred with high efficiency without perturbing the DNA duplex structure. PELDOR experiments revealed an exceptional narrow distance distribution, which enables identification of even small mismatch-induced structural changes of oligonucleotides. However, rigid spin labels may lead to a specific mutual orientation of both reporter groups. Therefore, the determination of interspin distance distributions under the assumption of an isotropic distribution of spin label orientations, as applied in common data analysis programs, may be inaccurate. Consequently, the PELDOR spectra and the derived distance distributions might be modulated by this effect. A study by Schiemann et al. using compound C as spin label, first introduced by Barhate et al., revealed that an accurate determination of distances and
relative orientations of spin centers in nucleic acids can be
achieved by orientation selective PELDOR experiments at X-
band frequencies.24
Here, we provide insight into conformational changes of
DNA caused by mismatch formation. To this end, orientation
selective PELDOR was used to examine the changes in the
structure and dynamics of fully matching as well as mispaired
spin labeled DNA duplexes. Mismatches were incorporated into
oligonucleotides containing nucleoside derivative 1 as reactive
group and the 4-azido TEMPO spin label 2 was introduced by
the click reaction. Four mismatched constructs with dT·dT and
da·da pairs located between two spin labeled positions (Table
1 and Scheme 1) were synthesized and analyzed. The results
reveal that changes of the interspin distances appear depending
on the type of the incorporated mismatches and nearest
neighbor environment.

Scheme 1. Spin Labeling of DNA by Click Reaction and
Formation of Duplexes Containing One dT·dT or da·da
Mismatch in Positions 8 or 9

Figure 1. Structures of 7-ethynyl-7-deaza-2′-deoxyadenosine and 4-
azido TEMPO conjugates.

Figure 2. Averaged PELDOR traces (9.4 GHz) measured at 50 K (see Materials and Methods for details). Left: background corrected dipolar evolution data, \( F(t) \); tick marks are separated by 0.05. Right: distance distributions, \( P(r) \), obtained by Tikhonov regularization (DeerAnalysis2008).30 Weak contributions from proton modulations are visible with a frequency of about 14.6 MHz. Data analysis and distance distributions are not significantly affected, as the proton modulation frequency would correspond to a distance of about 1.5 nm. To guide the eye, a dashed line is depicted at the position of the peak value of the perfectly matched DNA duplex 11·5 (\( r_{\text{peak}} \approx 1.83 \text{ nm} \)).
results and discussion

Introduction of Nitroxide Spin Labels and Mismatches into Oligonucleotides. A series of oligonucleotides was synthesized by solid-phase phosphoramidite synthesis, deprotected under mild deprotection conditions, and purified by reversed-phase HPLC. Ethynylated oligonucleotide strands 6, 7, and 10 were functionalized with 4-azido-2,2,6,6-tetramethyl-piperidine-1-oxyl (2) as reported previously using copper(I) catalyzed Huisgen–Sharpless–Meldal alkyne–azide cycloaddition (CuAAC). The formation of click products was confirmed by MALDI-TOF mass spectrometry and enzymatic hydrolysis (see Supporting Information, Table S1 and Figure S1). The spin labeled single strands were hybridized with unmodified second strands in solution of low salt concentration. 0.1 M NaCl, 10 mM MgCl2, and 10% glycerol yielded a set of duplexes with canonical base pairs (11-S) or containing T·dT or dA·dT mismatches (12-S, 11-S, 11-9, and 13-S). So, two bidentate dA·dT base pairs are located in the center of the duplex between two spin labeled positions. Tridentate dC·dG base pairs surround the spin labels at both sites (positions 6 and 11), thereby stabilizing the local structure. These positions were chosen to separate the impact of changes of the microenvironment of the spin label from those introduced by mispairings. Effects induced by mispairing and hybridization are further distinguished by investigation of double labeled single stranded (11) and double stranded DNA (11-S).

The formation of mismatches does not only cause changes of the thermodynamics and kinetics of a particular canonical base pair but also affects nearest neighbors. Consequently, the nearest neighbor model for Watson–Crick base pairs was extended to interactions between mismatches and neighboring base pairs. It was shown that with the exception of terminal positions the thermodynamics of a mismatch is independent of its position within a particular duplex but depends on the local environment of the mismatch. A thermodynamic analysis was performed for the perfectly matching and mismatched ethynylated duplexes (Table 1). For comparison, the \( T_m \) values of the duplexes containing one mismatched pair in between the spin labels are presented (Table 2). To be consistent with the conditions utilized in low temperature EPR experiments, 10% glycerol was added to the solution throughout all \( T_m \) measurements. As shown in Table 1, the replacement of two dA residues by 7-ethyl-7-deaza-2-deoxyadenosine (1) has a positive effect on duplex stability: the \( T_m \) value rises by 4 °C for 6-S compared to 4-S. However, the introduction of a dA·dT or dT·dT mismatch in between the ethynylated residues results into a pronounced duplex destabilization (\( \Delta T_m = -11 \)) in agreement with the former observation that the presence of dA·dT or dT·dT mismatches in the center of a duplex reduced the \( T_m \) value significantly. The introduction of the two spin labels into the perfectly matching DNA duplex (11-S) resulted in a \( T_m \) value of 41 °C (Table 2), which is only slightly decreased compared to the unmodified DNA duplex 4-S (\( T_m = 43 \) °C). This is evidence for a nearly undisturbed helix structure due to the uncritical position of the spin labels at C-7 of the 7-deazapurine moiety. The substitution of a dA·dT base pair by a dT·dT mispair (12-S) results into a pronounced duplex destabilization \( \Delta T_m = -12 \) °C compared to the perfectly matching DNA duplex 11-S. A similar result was obtained for duplex 11-S containing the dT·dT mismatch close to the second spin label toward the 5′-end of the duplex, \( \Delta T_m = -13 \) °C. Even more pronounced destabilizations were found in the case of the dA·dT mismatches. The \( T_m \) values are decreased by 17–19 °C (\( T_m = 22 \) °C for 11-S, \( T_m = 24 \) °C for 13-S).

Interspin Distance Changes Reveal Mismatch-Induced Conformational Changes. Misincorporations of nucleotides, if not repaired, have severe effects on the cellular machinery. Such alterations can lead to changes of the DNA conformation, which in turn may result in twists, bending, shortening, or elongation of the DNA double helix. PELDOR has been shown to provide valuable information on the impact of mispairings on the distances between spin labeled nucleotides. Siciolo et al. reported on interspin distance changes in duplex DNA induced by lesions. In these experiments, the two spin labels were located in the minor groove and separated by six nucleotides containing the lesion. Kuznetsov et al. presented experiments on DNA containing non-nucleotide inserts with spin labels bound at the 3′-end and 5′-end phosphate groups separated by 12 nucleotides and suggested a 20° bending of the double helix to occur. The large separation of the spin labels in these experiments favors the detection of lesion-induced global conformational alterations, which are accompanied by internucleotide distance changes along the double helix axis, i.e., shrinking, elongation, or 20° bending of the double strand. However, a large separation of the two spin labels from the lesion sites renders their distance value less sensitive to helix twists or only local conformational changes. In the present study, the two spin labels of the 12-mer duplex DNA are located in the major groove and separated by only two nucleotides. Hence, the present experiments are expected to be sensitive to local changes due to base pair overlaps or base pair twists, which could lead to alterations of interspin distances perpendicular to the helix axis.

PELDOR data depend on the mutual orientation of the spin label molecules. Orientation correlation can be neglected if the spin label side chains are sufficiently flexible. However, if the
Table 2. $T_m$ Values of Oligonucleotide Duplexes Containing Spin Labeled Conjugates* and Parameters for the Interspin Distance Distributions, $r_{peak}$ and $\sigma$, Determined by PELDOR Experiments

<table>
<thead>
<tr>
<th>duplex</th>
<th>$T_m$ (°C)</th>
<th>$\Delta T_m$ (°C)</th>
<th>$r_{peak}$ (nm)</th>
<th>$\sigma$ (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>4-5</td>
<td>43</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>41</td>
<td>-12</td>
<td>1.83 ± 0.02</td>
<td>0.5</td>
</tr>
<tr>
<td>11-5</td>
<td>29</td>
<td>-13</td>
<td>1.73 ± 0.02</td>
<td>0.5</td>
</tr>
<tr>
<td>12-5</td>
<td>28</td>
<td>-19</td>
<td>1.73 ± 0.03</td>
<td>0.7</td>
</tr>
<tr>
<td>13-5</td>
<td>24</td>
<td>-17</td>
<td>2.08 ± 0.03</td>
<td>0.7</td>
</tr>
</tbody>
</table>

*Measured at 260 nm in an aqueous 0.1 M NaCl solution containing 10 mM MgCl$_2$ and 10% glycerol with 5 μM single-strand concentration. $\Delta T_m$ was calculated as $T_m$(mismatched DNA duplex) − $T_m$(11-5). Error values were obtained utilizing the validation tool included in DEERAnalysis2008 (see Materials and Methods for details).

The observed increase or decrease of the interspin distances is strong evidence that the DNA mismatch site shrinks or expands. The resulting interspin distance changes depend not only on the type of mismatch (dA-dA or dT-dT) but even more pronounced on the mismatch position with respect to the spin labeled nucleotides as illustrated in Figure 3A,B. Hence, our experiments reveal that the mismatch-induced conformational changes are transmitted to the flanking nucleotides and that the impact of this mismatch depends on the nearest neighbor environment. Experimental NMR results revealed that DNA duplexes are not greatly distorted by the introduction of dA-dA or dT-dT mismatches and that their global conformation is that of a canonical B-form double helix in agreement with our finding. Furthermore, it was shown that, for both systems (dA-dA or dT-dT), two conformations with the same donor and acceptor pattern can coexist, which are engaged in rapid exchange, one being obtained from the other by a 180° rotation about the pseudodyadic axis (cf. Figure 3). Our experiments show that these local conformational changes affect in a position dependent manner the distance between nearest neighbors.

# CONCLUSIONS

This work reports on distance changes occurring in site-directed spin labeled duplex DNA when mismatches replace canonical base pairs. On the basis of ethynylated DNA, 4-azido TEMPO spin labels were introduced at the 7-position of the

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The Journal of Physical Chemistry B
MALDI-TOF mass data of oligonucleotides, enzymatic hydrolysis data, and Pake pattern. This material is available free of charge via the Internet at http://pubs.acs.org.

**ASSOCIATED CONTENT**

Supporting Information
MALDI-TOF mass data of oligonucleotides, enzymatic hydrolysis data, and Pake pattern. This material is available free of charge via the Internet at http://pubs.acs.org.

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**Notes**
The authors declare no competing financial interest.

**ACKNOWLEDGMENTS**

Financial support by the Volkswagenstiftung (I/79 950-952) (to D.W.) and by the ChemBiotech, Münster, Germany (to P.D.) is gratefully acknowledged.

**REFERENCES**

This paper was published on the Web on April 3, 2012, with the authors listed in an incorrect order. The corrected version was reposted on April 5, 2012.