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Spectroscopic Probe for the Detection of Local DNA Bending at an AAA Triplet†

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ABSTRACT: Spectroscopic evidence of a DNA bend in solution is presented by analyzing model 15-mer duplexes spin-labeled with the five-atom-tethered nitroxide DUAP located in the major groove. Three 15-mers containing AATT with DUAP enzymatically incorporated into three different positions yielded nearly identical line shapes while a fourth 15-mer containing AAATT produced an EPR spectrum with significant additional line broadening. These results are interpreted according to the dynamic cylinder model where the DNA dynamics are decoupled into overall and internal contributions. It is shown that the AAATT sequence induces a change in the internal dynamics characterized by local ordering of DUAP. The increase in ordering evident in 15-mers containing AAATT rather than AATT suggests that the former sequence gives rise to a bend toward the major groove resulting in spatial restriction of the probe.

The ability of DNA to adopt various conformations is crucial to the many cellular processes with which it is involved. For example, the formation of a bend in the arabinose operon has been found to repress the expression of the Ara proteins (Schleif, 1992). Furthermore, bending induced by protein binding appears to allow the formation of higher-order nucleoprotein complexes associated with transcription (Love et al., 1995; Jacobson & Tjian, 1996). DNA bending has been the subject of several reviews in recent years (Crothers et al., 1990; Hagerman, 1992; Sinden, 1994; Young et al., 1995a). It is believed that intrinsically bent or curved DNA results when some base sequences or structural motifs are repeated. Although most base sequences can give rise to DNA curvature, most bends are small compared with the effect produced by A-tracts. It has been found that polymers containing A_{4-9} repeated every 10 base-pair interval (one helical turn) are bent with the greatest bend occurring with A_6 (Koo et al., 1986). However, when the A-tract occurs in both strands, a sequence as short as A_3 results in bending as well (Hagerman, 1985). Global bending has been studied by gel electrophoresis (Crothers, 1994) as well as several other experimental methods including NMR and X-ray crystallography (Diekmann, 1992), but the structural bases for DNA bending are still unresolved (Zhurkin et al., 1991; Young et al., 1995b).

Spin-labeled nucleic acids have been used to detect the presence of various helical conformations. A variety of probes were designed for that purpose. We have focused on tethers varying in length (2−11 atoms) exhibiting different tether flexibilities (Kao et al., 1983; Kao & Bobst, 1985; Pauly et al., 1987; Strobel et al., 1990a, 1995; Keyes & Bobst, 1995), whereas others have used more rigid two- and four-atom tethers consisting of mono- and diacetylene linkages, respectively (Spaltenstein et al., 1989a; Hustedt et al., 1995). While the labels containing the propyne group cause a slight variation in the melting temperature with respect to the native duplex (Spaltenstein et al., 1988; Hustedt et al., 1995), such a perturbation has not been observed with the more flexible tethers. Actually, a systematic thermal

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denaturation study of a 26-mer labeled with either DUAP[1] or its six-membered ring analog DUAT revealed no difference in T_m between the labeled and unlabeled oligomers (Bobst et al., 1988). In addition, a preliminary study with the uvrABC protein complex showed that substitution of a four-atom-tethered nitroxide in position C5 of thymidine does not cause distortional sites in the duplex (Kao & Bobst, 1985). Although these two classes of spin labels display different spectroscopic characteristics, they have both been successfully used to monitor nucleic acid conformations. These include A-form RNA (Kao et al., 1983), B-form DNA (Kao & Bobst, 1985), Z-DNA (Strobel et al., 1990a, 1995), and hairpins (Spaltenstein et al., 1989b). While both classes of tether couple the nitroxide to the base, the experimental EPR line shapes will be significantly different for a given conformation. Even though each conformation displays a unique line-shape signature, the difficulty lies in interpreting these line shapes in terms of which motional modes are dominant and what their magnitudes are. Although the more flexible nonperturbing tethers employed by Bobst and coworkers may lead to an overestimation of local base motion, the acetylenic tethers may restrict the local base motion since it is believed that a propyne group is planar with respect to the heterocycle which could cause increased base stacking (Froehler et al., 1992).

EPR data have been successfully analyzed by using a "base disk" model which was applied to polymeric RNA and DNA systems (Kao et al., 1983; Kao & Bobst, 1985). The spin-labeled base diffusion is modeled as axially symmetric with the principal diffusion axis along the bond connecting the tethered nitroxide to the C5 base position. Rotation about this axis is characterized by $\tau_{||}$ which was found to depend upon tether length. Motions perpendicular to the principal diffusion axis are quantified by $\tau_{\perp}$ and were found to be dependent upon duplex conformation and independent of tether length. A consistent value of about 4−6 ns for $\tau_{\perp}$ was found for B-form nucleic acid polymers (Bobst et al., 1984; Keyes & Bobst, 1995), while the value was approximately doubled for Z-DNA (Strobel et al., 1990a,b). From preliminary studies of a B-DNA 26-mer and polymer spin-labeled with the five-atom-tethered nitroxide DUAT, it was concluded that the 4 ns value is also independent of the helix length (Bobst et al., 1988). At that time, the detectable small difference between the spectral line shapes of the oligomer and polymer was attributed to a small change in tilt angle due to some subtle geometric difference in the major groove of the two systems. However, a more recent systematic study on a series of nucleic acids of varying length (15-mer, 30-mer, 45-mer, and polymer) indicates that there is some helix length dependence detected by $\tau_{\perp}$ (1.4−6.2 ns) (Keyes & Bobst, 1995). Thus, $\tau_{\perp}$ is sensitive to both internal and global dynamics.

These two classes of motion are decoupled in the "dynamic cylinder" model (Keyes & Bobst, 1995). This model was inspired by a theoretical framework published by Hustedt et al. (1993a). Both the base disk and dynamic cylinder models are special cases of the more general slowly relaxing local structure (SRLS) model (Polimeno & Freed, 1995). The essence of the dynamic cylinder model is that it describes a diffusing hydrodynamic cylinder (e.g., DNA helix) where the principal diffusion axis corresponds to the long axis of the cylinder. The internal dynamics consisting of spin-label motion, base oscillations, and collective bending and twisting modes are characterized by an order parameter *S* which is determined by linear averaging of the hyperfine tensor elements (Keyes & Bobst, 1995). Calculating *S* by this simple model yields a measure of motional restriction of the spin-labeled base. For instance, in the case of a B to Z transition monitored by the five-atom-tethered DCAT, the *S* value increased from 0.15 to 0.26 (Keyes & Bobst, 1995). This increase indicates a local ordering which is consistent with studies claiming that Z-DNA is more rigid than B-DNA (Hagerman, 1988). Hustedt et al. (1993a) used the tensor averaging models of Van et al. (1974) to calculate mean squared amplitudes of internal motion which can be used to calculate a dynamic flexural persistence length.

FIGURE 1: Oligomer sequences containing the DUAP spin label in each of four positions. The label positions are numbered with respect to the dyad axis.

In this paper, we present spectroscopic evidence of a sequence-specific DNA bend in solution. The five-atom-tethered spin label DUAP was enzymatically incorporated into three model DNA 15-mers containing AATT and a fourth containing AAATT (Figure 1). The 15-mer containing the triplet AAA yielded a spectral line shape with significant broadening with respect to the spectra of the other three oligomers. The spectra were simulated according to the dynamic cylinder model (Keyes & Bobst, 1995). The triplet A-tract sequence yielded an order parameter of 0.32 compared with a value of 0.20 for the other three oligomers indicating greater motional restriction of DUAP. This

[1] Abbreviations: CD, circular dichroism; DCAT, 5-[3-(2,2,6,6-tetramethyl-1-oxypiperidine-4-carboxamido)prop-1-enyl]-2′-deoxycytidine; DUAP, 5-[3-(2,5-dihydro-2,2,5,5-tetramethyl-1-oxyl-1H-pyrrole-3-carboxamido)prop-1-enyl]-2′-deoxyuridine; DUAT, 5-[3-(2,2,6,6-tetramethyl-1-oxypiperidine-4-carboxamido)prop-1-enyl]-2′-deoxyuridine; EPR, electron paramagnetic resonance; τ_{rb}, dynamic cylinder rigid-body correlation time; $T_{||}$, parallel component of τ_{rb}; $T_{\perp}$, perpendicular component of τ_{rb}; *S*, order parameter.

Table 1: Simulation Parameters Used To Determine *S* for 15-mer Sequences

compound	g'_{xx}	g'_{yy}	g'_{zz}	A'_{xx}	A'_{yy}	A'_{zz}	S	$T_{\|\|}$	$T_{\perp}$	θ_{dc}	B
4-DUAP 15-mer	2.0071	2.0060	2.0055	12.2 G	15.9 G	20.0 G	0.20	4.7 ns	11.4 ns	50°	1.01 G
3-DUAP 15-mer	2.0071	2.0060	2.0055	11.1 G	14.4 G	22.4 G	0.32	4.7 ns	11.4 ns	50°	0.97 G

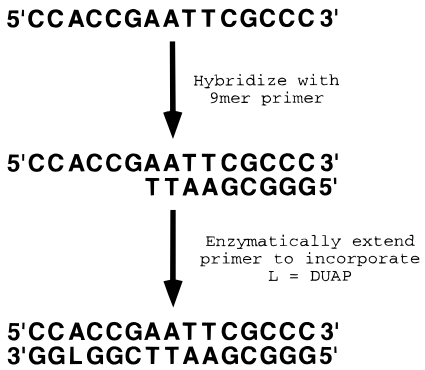

FIGURE 2: Synthesis scheme for DUAP-labeled 15-mers.

restriction appears to result from the triplet AAA induced narrowing of the major groove due to bending.

MATERIALS AND METHODS

Synthesis of Spin-Labeled Oligomers. The four DNA oligomers were synthesized by first hybridizing the 9-mer sequence GGGCGAATT to each of the 15-mer sequences CCACCGAATTCGCCC, CCCACGAATTCGCCC, CCCCA-GAATTCGCCC, and CCCCGAAATTCGCCC. The 9-mer and 15-mers were produced on an Applied Biosystem (Foster City, CA) 380A DNA Synthesizer using phosphoramidite chemistry. Purification of the products was achieved by Bio-Gel P-2 (Bio-Rad Laboratories, Richmond, CA) column chromatography and FPLC on an anion-exchange MonoQ column (Pharmacia Inc., Piscataway, NJ). 1.4 nmol of 15-mer with 1.3 nmol of 9-mer were annealed overnight at 4 °C in propylene tubes. The Klenow fragment was then used to fill in the remaining sequence with dCTP, dGTP, and DUAP (Figure 2). The filling reaction was carried out with 25 nmol of pppDUAP, 140 nmol of dGTP, and 25 nmol of dCTP in a total volume of 260 μL with a final concentration of 0.14 M Tris-HCl, 0.05 M NaCl, pH 7.8, 0.014 M $MgCl_2$, 1.4 mM DL-dithiothreitol, and 28 μg/mL bovine serum albumin in the presence of 25 units of Klenow fragment (Promega, M220) at 25 °C. The enzymatic reaction was stopped after 160 min with 50 μL of 0.3 M EDTA. About 0.8 nmol of double-stranded DUAP-labeled 15-mers could be isolated by this method. The 15-mers were purified by Bio-Gel P-30 gel filtration using as elution profile 0.04 M NH_4HCO_3, pH 7.5, and 10% ethanol. An FPLC analysis of the product gave two peaks of which only the one eluting at the higher salt concentration gave an EPR signal.

EPR Spectra and Simulations. The EPR spectra were recorded on a Bruker ESP 300 spectrometer fitted with a TM cavity. The instrument parameters were: modulation amplitude 1.6 G; modulation frequency 100 kHz; microwave power 32 mW; sweep width 100 G; conversion time 164 ms; time constant 164 ms; and 9 scans. The EPR data were obtained on a sample volume of 190 μL containing 0.2 nmol of DUAP-labeled 15-mers in 0.01 M K_2HPO_4, pH 7, 0.1 M NaCl, and 1 mM EDTA.

The spectra of all four 15-mers were simulated according to the dynamic cylinder model (Hustedt et al., 1993a; Keyes & Bobst, 1995) using an axially symmetric simulation program (Schneider & Freed, 1989) coupled with a nonlinear fitting routine (Hustedt et al., 1993b; Keyes & Bobst, 1995). A cylinder hydrodynamic model (Tirado & García de la Torre, 1980) was used to describe the diffusion of the DNA helix. The principal magnetic axis through the $2p_z$ orbital of the N of the nitroxide is at an angle θ_{dc} [note θ_{dc} corresponds to θ_{tilt} in Hustedt et al. (1993a)] with respect to the principal diffusion axis of the cylinder.

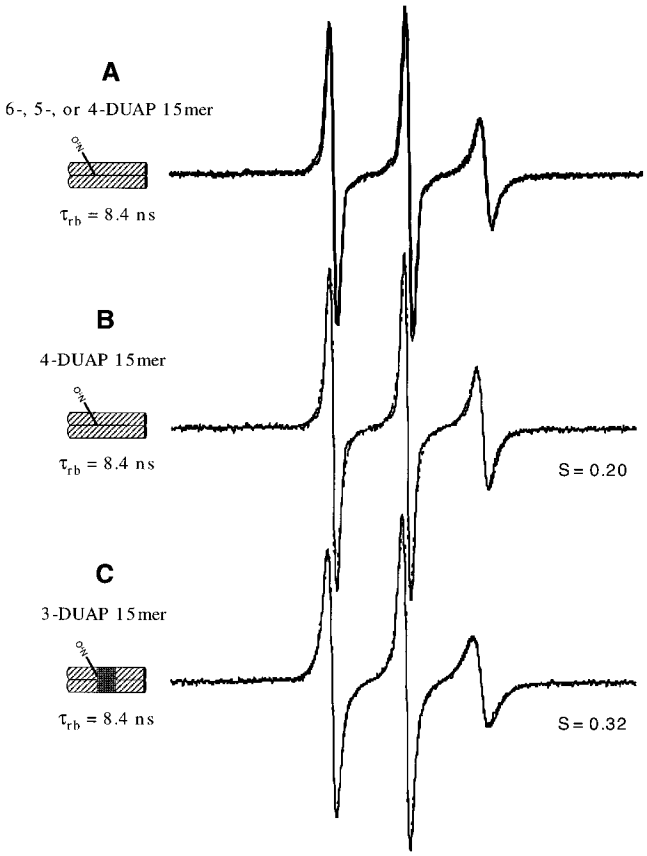

FIGURE 3: Spin-labeled oligomers with corresponding EPR spectra. The 15-mers are represented as hydrodynamic cylinders with the same global diffusion rate characterized by τ_{rb}. (A) Superimposed experimental EPR spectra of 6-, 5-, and 4-DUAP 15-mers display nearly identical line shapes. Experimental (solid line) and simulated (dashed line) spectra of the (B) 4-DUAP 15-mer and (C) 3-DUAP 15-mer illustrate the line-shape change observed for the triplet AAA sequence where the base-pair step conformational change is indicated by shading.

Molecular Model. The bent B-DNA 12-mer coordinates were obtained from the Nucleic Acid Database (Index #BDL011). The DUAP spin label was constructed in the Builder module of Insight II (MSI) and attached to the 12-mer in position 3 with respect to the dyad axis. The canonical B-DNA structure was built with the Biopolymer module. The ribbon traces were generated in the Viewer module.

RESULTS

The experimental and simulated EPR spectra of the four oligomers are illustrated in Figure 3. Figure 3A displays

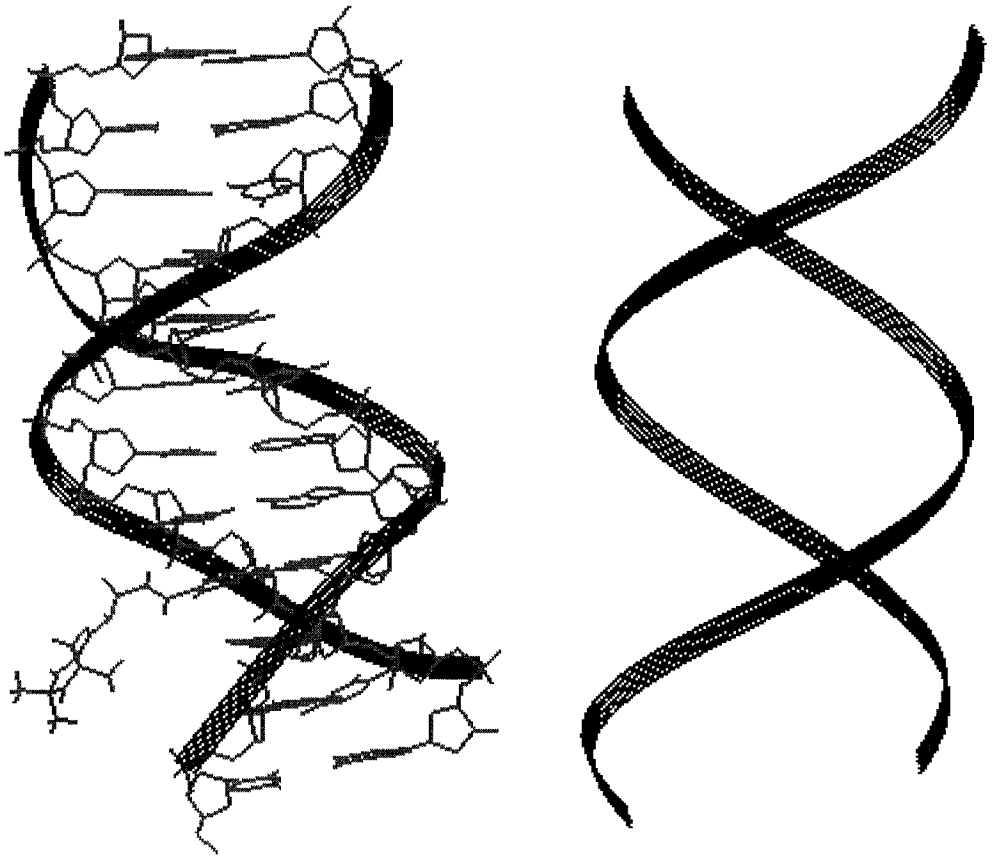

FIGURE 4: Molecular model (NDB BDL011) containing DUAP (L) in position 3 resulting in the sequence $^{5'\text{-CGCAAATTCGCG-3'}}_{3'\text{-GCGLTTAAGCGC-5'}}$ with backbone ribbon shown (left) to illustrate structural distortion with respect to canonical B-DNA 12-mer (right).

Table 2: Rotational Invariance of Magnetic Tensors

compound	$\bar{g}$	$^1/_3$Tr $\boldsymbol{g}$	$^1/_3$Tr $\boldsymbol{g}'$	$\bar{A}$	$^1/_3$Tr A	$^1/_3$Tr A′
4-DUAP 15-mer	2.0062	2.0062	2.0062	16.1 G	16.0 G	16.0 G
3-DUAP 15-mer	2.0062	2.0062	2.0062	16.1 G	16.0 G	16.0 G

the EPR spectra of the AATT-containing 15-mers labeled either in position 4, 5, or 6 which are essentially superimposable. The experimental spectrum of the 4-DUAP 15-mer is shown in Figure 3B along with the simulated spectrum yielding an order parameter $S = 0.20$. The 3-DUAP 15-mer yields a significantly broadened experimental spectrum as shown in Figure 3C. A computer simulation of this spectrum is also shown in Figure 3C with a value $S = 0.32$. Table 1 lists the parameters used to simulate the EPR spectra, and Table 2 displays the rotational invariance of the **g** and **A** tensors, confirming the consistency of the motional averaging. Also, as can be seen from Table 1, the hydrodynamic cylinder correlation times are held constant from which the rigid body correlation time $\tau_{rb} = 8.4$ ns can be calculated (Keyes & Bobst, 1995).

Figure 4 illustrates ribbon traces of both the 12-mer $^{5'\text{-CGCAAATTCGCG-3'}}_{3'\text{-GCGLTTAAGCGC-5'}}$ spin-labeled in position 3 with DUAP (L) and an unlabeled canonical B-DNA 12-mer. The distorted B-DNA structure on the left is from X-ray data of a DNA 12-mer with an 18° bend (Drew & Dickerson, 1981). An increase of 1.8 Å was calculated for the cross-section perpendicular to the cylinder axis of the bent structure compared to the canonical B-DNA. Based on Figure 6 of García de la Torre and Bloomfield (1981), the components of the global diffusion tensor of a bent rod change as a function of the degree of bending. However, in view of the small change in duplex dimension due to the bend illustrated in Figure 4, it is assumed as a first approximation that the cylinder correlation times are the same for all four oligomers (Table 1).

DISCUSSION

The sensitivity of a five-atom-tethered spin label in detecting a change in nucleic acid conformation was demonstrated with DCAT, a cytidine analog of DUAP (Strobel et al., 1990a,b; Keyes & Bobst, 1995). The EPR spectrum of $(dG\text{-}dC)_n$ spin-labeled with DCAT showed significant line broadening when placed in 4.5 M NaCl. CD confirmed that the effect was the result of a B to Z conformational transition. That the EPR broadening could be attributed to a nucleic acid conformational change and not just to a viscosity change of the solution was verified by running control experiments with spin-labeled $(dA\text{-}dT)_n$. $(dA\text{-}dT)_n$ is known not to undergo a salt dependent B to Z transition, and indeed no EPR line-shape change was observed between the low- and high-salt EPR spectra (Strobel et al., 1990b). A more recent motional analysis of the B and Z spectra indicates that the order parameter increases from 0.15 to 0.26 suggesting greater rigidity in the Z form (Keyes & Bobst, 1995).

In this paper, we present experimental data that conclusively show that in a series of 15-mers a five-atom-tethered spin-labeled base reports a sequence-dependent motional effect. The global motion of all four 15-mers is assumed to be very similar, and the sequence-dependent line-shape change is attributed to an increase in the ordering of DUAP. No attempt is made to analyze the experimental findings in terms of a particular geometry or amplitude of motion.

DUAP allows the detection of subtle conformational changes that occur from one base pair to the next as a result of ordering of the probe. The increase in order evident in the base-pair step from position 4 to 3 indicates a structural distortion at this point in the sequence.

Such base-pair steps have been implicated in the bending process (Young et al., 1995b). Based upon studies of oligomers containing AAA triplets (Hagerman, 1985), this structural distortion appears to correspond with a bend. Furthermore, crystallographic studies have found that AA steps result in compression of the major groove (Young et al., 1995b). Figure 4 is shown to illustrate the distortions present in an AAA triplet-containing sequence when compared with canonical B-DNA. In view of the location of the tethered nitroxide in the major groove (Figure 4), the observed change in the EPR line shape can be ascribed to an increased ordering of the probe due to a narrowing of the major groove.

While gel electrophoresis has been effective for the study of global bending when the sequence is long enough and the bend is prominent, and X-ray studies on crystals have shown the effect of sequence upon bending, the spectroscopic probe DUAP allows the detection of sequence-dependent local distortions in solution that form the basic building blocks of bending. One aspect of bending currently under discussion is which individual base-pair steps induce a roll toward the minor or major groove (Zhurkin et al., 1991; Young et al., 1995b). The increased ordering observed with DUAP in AAATT implies that the bend occurs toward the major groove in this sequence.

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