Overall and Internal Dynamics of DNA as Monitored by Five-Atom-Tethered Spin Labels

Robert S. Keyes, Elisabeth V. Bobst, Ying Y. Cao, and Albert M. Bobst
Department of Chemistry, University of Cincinnati, Cincinnati, Ohio 45221 USA

ABSTRACT Electron paramagnetic resonance (EPR) spectra of the two-atom-tethered six-membered ring thymidylate spin label (DUMTA) incorporated into duplexes of different sizes were found to display a helix length dependence and a local-order parameter $S = 0.32 \pm 0.01$ for B-DNA based on the dynamic cylinder model (Keyes, R. S., and A. M. Bobst. 1995. Detection of internal and overall dynamics of a two-atom-tethered spin-labeled DNA. *Biochemistry*. 34:9265–9276). This sensitivity to size, which reflects global tumbling, is now reported for the more flexible five-atom-tethered five-membered ring thymidylate spin label (DUAP) that can be readily incorporated enzymatically and sequence specifically into nucleic acids of different sizes. The DUAPs containing B-DNA systems were simulated with the same dynamic cylinder model, giving $S = 0.20 \pm 0.01$ for the more flexibly tethered spin label. This shows that S is dependent on tether length but not on global motion. An analysis with the same motional model of the B-Z transition in a $(dG\text{-}dC)_n$ polymer containing the five-atom-tethered six-membered ring cytidylate spin label (DCAT) (Strobel, O. K., R. S. Keyes, and A. M. Bobst. 1990b. Base dynamics of local Z-DNA conformations as detected by electron paramagnetic resonance with spin-labeled deoxycytidine analogues. *Biochemistry*. 29:8522–8528) revealed an increase in S from 0.15 ± 0.01 to 0.26 ± 0.01 in response to the B- to Z-DNA transition. This indicates that S is not only sensitive to tether length, but also to conformational changes in DNA. Both the DUAP- and the DCAT-labeled systems were also simulated with a base disk model. From the DUAP spectral series, the perpendicular component of the correlation time $\tau_\perp$ describing the spin-labeled base diffusion was found to be sensitive to global tumbling, confirming earlier results obtained with DUMTA. The DCAT polymer results demonstrated that $\tau_\perp$ monitors a conformational change from B- to Z-DNA, indicating that $\tau_\perp$ is also sensitive to local base dynamics. These results confirm that the dynamics of five-atom-tethered nitroxides are coupled to the nucleic acid dynamics and, as with two-atom-tethered spin labels, can be characterized by S and $\tau_\perp$. The analyses of both spin-labeled systems provide good evidence for spin-labeled base motions within double-stranded DNA occurring on the nanosecond time scale, and establish that both labels can be used to monitor changes in global tumbling and local order parameter due to variations in DNA conformation and protein-DNA interactions.

INTRODUCTION

DNA is a highly dynamic molecule possessing a number of motional components. Although there is still much conjecture, these motions may serve various functions in protein recognition (Hogan and Austin, 1987), gene control (Burd et al., 1975a,b; Early et al., 1977; Hogan et al., 1979; Ptashne, 1986; Schleif, 1988; Sullivan et al., 1988), and the development of nucleic acid tertiary structure (Cantor et al., 1988; Kanaar and Cozzarelli, 1992; Kim et al., 1993). Apart from the possible functions that dynamics within DNA may serve, the observation that specific nucleic acid conformations are associated with distinguishing sets of dynamics that serve as signatures indicates that analyses of these motions are useful for identifying particular conformations as well (Bobst, 1979; Bobst et al., 1975, 1996a; Kao and Bobst, 1985; Kao et al., 1983; Spaltenstein et al., 1989a,b; Strobel et al., 1990a,b, 1995).

Investigations into nucleic acid dynamics have developed in the Bobst group in conjunction with two lines of research, both using EPR spectroscopy. The first line of work involves monitoring the dynamics of protein-nucleic acid complexes. This is accomplished by placing spin labels either inside the protein binding site, as with polylysine (Bobst et al., 1985) and gene 5 single-strand binding protein (Kao et al., 1985), or outside the binding site, as in the cases of the site-specific *Eco*RI endonuclease (Keyes et al., 1996) and the homeodomain (Bobst et al., 1996b). By placing the labels at various distances from the binding site, the transmission of structural information can be monitored. By linking spin-labeled nucleic acid dynamics to protein binding through EPR competition experiments, binding affinities of single-strand binding proteins can be determined as well (Keyes and Bobst, 1993).

The second area of research entails monitoring conformational changes within nucleic acids. It has been possible for many years to detect the single-strand to double-strand interconversion (Bobst, 1979; Bobst et al., 1975). One application where this proves useful is the development of hybridization probes (Strobel et al., 1991; Murakami et al., 1993). A short nucleic acid sequence containing a spin label can be constructed to determine the presence or absence of the complementary sequence within a much larger stretch of RNA or DNA. In addition, spin labeling enables detection of the B-Z transition (Strobel et al., 1990a,b; 1995) and DNA bending (Bobst et al., 1996a).

How well the spin label reflects DNA motions is a property of the tether. The better the coupling between the

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Address reprint requests to Dr. Albert M. Bobst, Department of Chemistry, University of Cincinnati, ML 172, Cincinnati, OH 45221-0172. Tel.: 513-556-9281; Fax: 513-556-9239; E-mail: albert.bobst.@uc.edu.

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nitroxide and the base to which it is attached, the closer the nitroxide dynamics will reflect the base dynamics. Although spin labels with long tethers (i.e., 5–11 atoms in length) are not as well coupled as those with shorter tethers, they still consistently detect the magnitude of base motion (Strobel et al., 1990b, 1995). In addition, they have the advantage of being acceptable substrates for enzymatic incorporation into nucleic acids, whereas the short-tethered nitroxides (i.e., 1–2 atoms) do not incorporate well (Toppin, 1983). Furthermore, a UV melting study of DNA 26mers spin-labeled with five-atom-tethered nitroxides indicated no destabilization of the helix based upon the T_m value (Bobst et al., 1988). Both labeled and unlabeled oligomers produced identical melting curves. Study of the susceptibility of spin-labeled sites to enzymatic repair using the uvrABC protein complex showed that substitution of a five-atom-tethered nitroxide in position C5 of thymidine does not cause distortional sites in the duplex (Kao and Bobst, 1985).

Another class of spin labels consisting of mono- and diacetylene-tethered nitroxides has been developed by Robinson and co-workers to more rigidly couple nitroxide dynamics to DNA dynamics (Spaltenstein et al., 1988; Kirchner et al., 1990). The monoacetylene spin label was used to detect the presence of a hairpin conformation (Spaltenstein et al., 1989a) and to determine the dynamic persistence length of DNA (Hustedt et al., 1993a). When monoacetylenic tethers are used, the EPR spectra are predominantly slow-motional, and it was concluded that there is no significant ~4 ns (Spaltenstein et al., 1989b) or rapid, large amplitude (Hustedt et al., 1993a, 1995) base motion. On the other hand, evidence for substantial base dynamics has been found from crystallography (Holbrook and Kim, 1984; Klimasauskas et al., 1994), EPR (Strobel et al., 1990b, 1995), fluorescence (Nordlund et al., 1989; Guest et al., 1991; Georghiou et al., 1996), and NMR (Leroy et al., 1988; Eimer et al., 1990; Folta-Stogniew and Russu, 1994; García de la Torre et al., 1994). In fact, a recent study monitoring the intrinsic fluorescence of thymine argues for the existence of large-amplitude base motions in DNA (Georghiou et al., 1996). Froehler et al. (1992) have demonstrated that the presence of a propyne group in the C5 position of pyrimidine bases stabilizes the DNA duplex. This may explain the reduced base motion observed with propyne-containing spin labels (Spaltenstein et al., 1989b; Hustedt et al., 1993a, 1995). Examination of the melting profile of a monoacetylene-labeled dodecamer indicates both hypochromic stabilization of the helix before the melting transition and an increase in T_m value (Spaltenstein et al., 1988). Although a diacetylene-tethered spin label yielded a motionally narrowed spectrum, analysis of the data indicated that rapid, large-amplitude local base dynamics were not present (Hustedt et al., 1995). The propyne stabilization observed with the monoacetylenic label may also be the reason for the small base motion detected by the diacetylenic label.

The theoretical framework developed for interpreting the dynamics of nucleic acids spin-labeled with a two-atom-tethered six-membered ring nitroxide (DUMTA) (Keyes and Bobst, 1995) is applied here to five-atom-tethered systems containing five- (DUAP) and six- (DCAT) membered rings (Fig. 1). The consistency between the results for two- and five-atom-tethered spin labels studied by this approach is found to be excellent and demonstrates that these labels are coupled to DNA dynamics. The global motion for all spin-labeled systems is well modeled as the diffusion of a hydrodynamic cylinder verifying the helix length dependence of the EPR spectra. In the case of polymers, the cylinders are assumed to be diffusing in the rigid limit. Analysis of the internal dynamics extends earlier work by demonstrating that the local-order parameter S is sensitive to conformational changes, suggesting that DNA conformation is linked to base amplitude. Simulation of spin-labeled polymers indicates that the rate of spin-labeled base motion in double-stranded DNA occurs on the nanosecond time scale.

MATERIALS AND METHODS

Spin-labeled DNA

The spin-labeled DNA molecules were constructed according to procedures developed in the Bobst laboratory. The DUAP {i.e., 5-[3-(2,5-dihydro-2,2,5,5-tetramethyl-1-oxyl-^{1}H-pyrrole-3-carboxamido)prop-1-enyl]-2′-deoxyuridine}-labeled *Eco*RI 15mer was made by hybridizing the 9mer sequence GGGCGAATT to the 15mer sequence CCCCAGAAT-

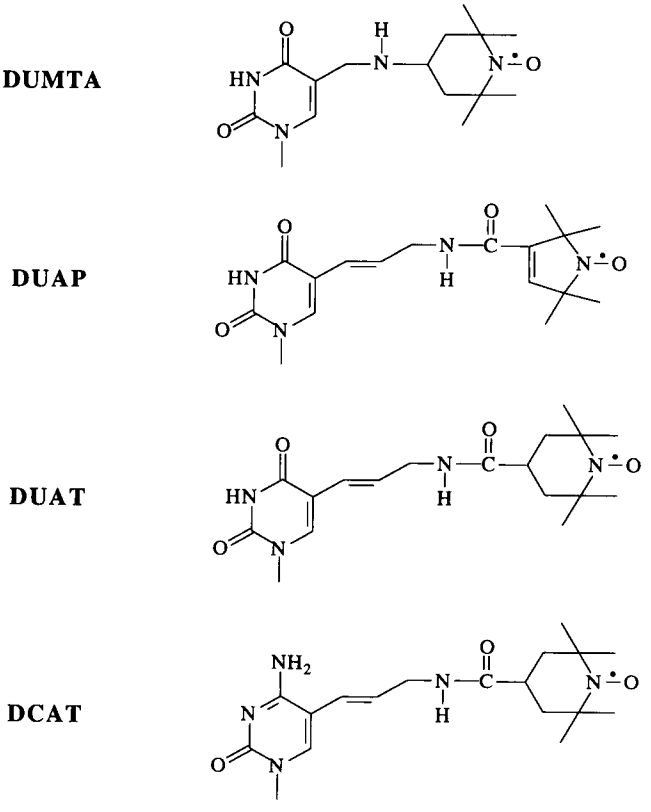

FIGURE 1 The chemical structures of the four spin labels discussed in the text.

TCGCCC, and filling out the sequence using the Klenow fragment and mononucleotides G, C, and DUAP (Bobst et al., 1988). Similarly, the 26mer was synthesized by making a $(C)_4A(C)_3GCGAATTCG$ sequence and hybridizing the 17mer with itself, resulting in a fragment with 8 basepairs and 9 base sticky ends, which were enzymatically filled. A comparable procedure was used to generate the 52mer starting with the 30mer sequence $(C)_{17}A(C)_3GCGAATTCG$. The spin label is in position 4 with respect to the dyad axis of the GAATTC sequence for the 15mer, and in position 9 for the 26mer and 52mer. The spin-labeled (dT, DUAP)$_n$·(dA)$_n$ polymer was prepared by enzymatically incorporating DUAP into (dT)$_n$·(dA)$_n$ with Pol I (Ireland et al., 1986; Toppin et al., 1986). The DCAT {i.e., 5-[3-(2,2,6,6-tetramethyl-1-oxypiperidine-4-carboxamido)prop-1-enyl]-2′-deoxycytidine}-labeled (dG-dC)$_n$ polymer was synthesized by nick translation using Pol I/DNase I, and the B to Z transition was induced by 4.5 M NaCl (Strobel et al., 1990a,b). All EPR spectra were measured on a sample volume of 190 μl containing 0.2 to 0.5 OD_{260}/ml of DNA material with a Bruker ESP 300 spectrometer. Instrument parameters were: modulation amplitude 1 G; modulation frequency 100 kHz; microwave power 10 mW; sweep width 100 G; conversion time 164 ms; time constant 164 ms; and receiver gain 8×10^5.

EPR simulations

All simulations were performed with the EPRFIT nonlinear fitting program (Hustedt et al., 1993b) as discussed in Keyes and Bobst (1995). The dynamics of a base result from a motional buildup consisting of components from both internal dynamics and global tumbling (Keyes and Bobst, 1995). The many motional modes can be subsumed into two classes of motions: internal dynamics and global dynamics. Keyes and Bobst (1995) found that the empirical proportionality:

$$\tau_\perp \propto S^2\tau_{rb} \tag{1}$$

provides a base-helix correlation between the base disk and dynamic cylinder motional models. $\tau_\perp$ is the perpendicular component of the base disk correlation time, τ_{rb} is the rigid-body correlation time for global tumbling of the DNA helix, and S is an order parameter representing motional restriction of the internal dynamics. The values for the three variables are obtained from base disk simulations ($\tau_\perp$), dynamic cylinder simulations (S), and hydrodynamic theory (τ_{rb}) (Keyes and Bobst, 1995—note that in Eq. 3 of this earlier paper, the exponent on R should be 2 rather than 3). τ_{rb} is a function of the cylinder diffusion coefficients $D_\parallel$ and $D_\perp$ ($T = 1/6D$) calculated according to Tirado and García de la Torre (1980).

RESULTS

DUAP spectra

The experimental EPR spectra of the DUAP-labeled 15mer, 26mer, 52mer, and polymer are displayed in Fig. 2, illustrating their sensitivity to the helix length. The decrease in global tumbling rate of the oligomers as helix length increases is effectively modeled by the hydrodynamic cylinder equations implicit in the dynamic cylinder model (Fig. 3 and Table 1). The base disk simulations demonstrate that the spin-labeled base diffusion is sensitive to the reduction in global diffusion as the DNA helix gets longer (Table 2).

DCAT spectra

The dynamic cylinder and base disk simulation parameters are tabulated in Tables 1 and 2, respectively.

Consistency of simulation parameters

For the dynamic cylinder model, the averaged set of **g** and **A** tensors are nearly constant for a given spin label and DNA conformation as the correlation times are changed according to the Tirado and García de la Torre equations (Keyes and Bobst, 1995) (Table 1). In addition, Tr **A**′ and Tr **g**′ are approximately equal to Tr **A** and Tr **g**, respectively, for each of the spin-labeled molecules, in agreement with the fact that the trace of a second-order tensor is rotationally invariant (Table 3). This indicates that the change in the magnetic tensors is the result of motional, and not polarity, effects.

Three different-order parameters were calculated for each spin-labeled molecule (Table 4). S (Eq. 8, Keyes and Bobst, 1995) was preferred, because it takes into account all three diagonal elements of the hyperfine tensor. S_z, defined with respect to the z-components (Eq. 9, Keyes and Bobst, 1995), is equivalent to S for these systems. S_g, obtained by substituting g-values for A-values in Eq. 9 of Keyes and Bobst (1995), provides another consistency test. The values shown in Table 4 demonstrate that both S and S_g provide similar order parameters. The variation in order parameter between DCAT ($S = 0.15 \pm 0.01$) and DUAP ($S = 0.20 \pm 0.01$) may be due to the difference in base. Changes in order parameter for a specific spin label are used for characterizing internal dynamics.

For the base disk model, $\tau_\parallel$ is representative of the particular tether length of the spin label and is kept constant. The value of $\tau_\parallel$ for the six-membered ring DUMTA is in good agreement with what is expected for spin labels based upon results obtained with other tether lengths (Fig. 4 *A*). As the tether length increases, the value of $\tau_\parallel$ for six-membered ring nitroxides decreases approximately linearly. The observation that both six- and eleven-atom tethers are well described by $\tau_\parallel = 0.06$ ns can be explained by earlier results indicating that the spin-label nitroxide ring is moved beyond the major groove into the bulk environment upon changing the tether length from five to six atoms (Bobst et al., 1984). It seems that beyond a tether length of six atoms, $\tau_\parallel$ remains nearly constant. Note that DUAP contains a five-membered ring which, possibly due to a change in local water structure, probably accounts for $\tau_\parallel = 0.1$ ns compared with DCAT, with its six-membered ring nitroxide, which yields $\tau_\parallel = 0.2$ ns. Clathrate-type cages have been found to alter the rate of spin-label diffusion from the expected hydrodynamic values (Goldman et al., 1972).

The order parameter mirrors the change in $\tau_\parallel$ as expected (Fig. 4 *B*). As the tether length increases, there will be an increase in nitroxide motion. However, even for a long tether of 11 atoms, the nitroxide is coupled to the base (Strobel et al., 1995).

The values of the tilt angles for the dynamic cylinder ($\theta_{dc} \cong 50°$) and base disk ($\theta_{bd} = 40°$) models used to simulate the five-atom-tethered systems correlate well with those used for the two-atom-tethered spin-label DUMTA (Tables 1 and 2).

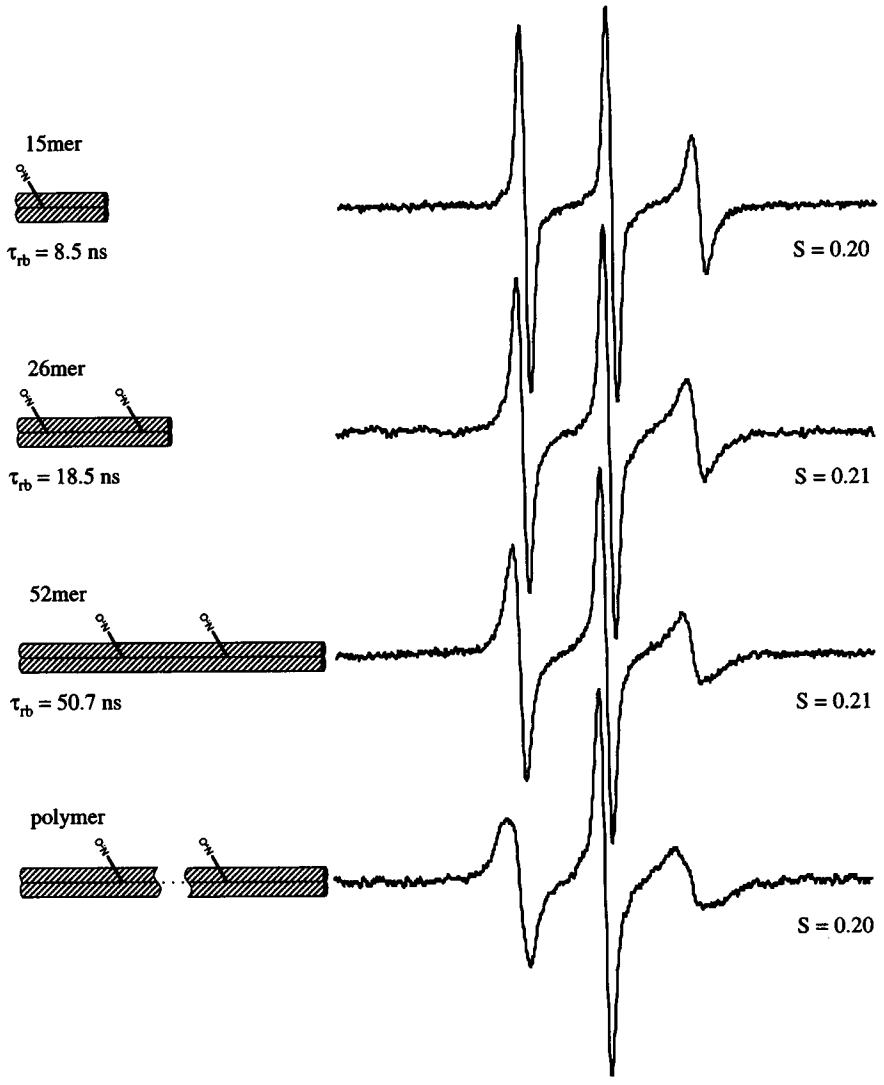

FIGURE 2 Experimental EPR spectra of DUAP-labeled duplexes demonstrating the sensitivity of DUAP to helix length. The spectra were recorded at room temperature in a 0.1 M NaCl, 0.01 M K_2HPO_4, 0.001 M EDTA, pH 7.0 buffer for the oligomers and a 0.01 M NaCl, 0.01 M sodium cacodylate, 0.001 M EDTA, pH 7.2 buffer (Ireland et al., 1986) for the polymer.

Correlation between the two models

To confirm the effect observed with the two-atom-tethered DUMTA-labeled molecules (Keyes and Bobst, 1995), the helix length dependence of $\tau_\perp$ was compared to the helix length dependence of both τ_{rb} and $S^2\tau_{rb}$ (Fig. 5) for the five-atom-tethered DUAP-labeled oligomers. When τ_{rb} is weighted by the square of the order parameter (Fig. 5 *B*), the slope is similar to that of $\tau_\perp$. The fact that the two lines in Fig. 5 *B* display a similar dependence on helix length supports the proportionality of Eq. 1, and illustrates that S remains nearly constant for oligomers of different lengths containing a given spin label.

The base-helix correlation concept was derived in Keyes and Bobst (1995). It has similarities to a relationship developed by Eimer et al. (1990) based upon the model-free approach of Lipari and Szabo (1982). The model-free approach is not directly applicable to EPR, but the $S^2\tau_{rb}$ term also appears in spectral density equations for EPR spectra [for instance, see Eq. 7.22 of Polnaszek and Freed (1975) and Eq. 17 of Zager and Freed (1982)] and represents the component of global motion contributing to the spectral density according to the magnitude of S. It must be emphasized that the base-helix correlation concept is an empirical relation, the merit of which is verified in both Keyes and Bobst (1995) and the present paper. Its applicability to physical systems is demonstrated by its usefulness in correlating the dynamic cylinder and base disk models for both two- and five-atom-tethered spin-labeled oligomers.

DISCUSSION

The detection of internal and global DNA dynamics by EPR can be achieved by attaching a nitroxide ring to the C5 position of thymidine or cytidine by a tether. It has been found that the nature of the tether is critical in the design of a spin label that is coupled to the base motions, but does not perturb those motions. Robinson and co-workers have worked with mono and diacetylenic tethers, of two and four atoms in length, respectively, to rigidly couple the nitroxide to the DNA motions. The Bobst group has used more

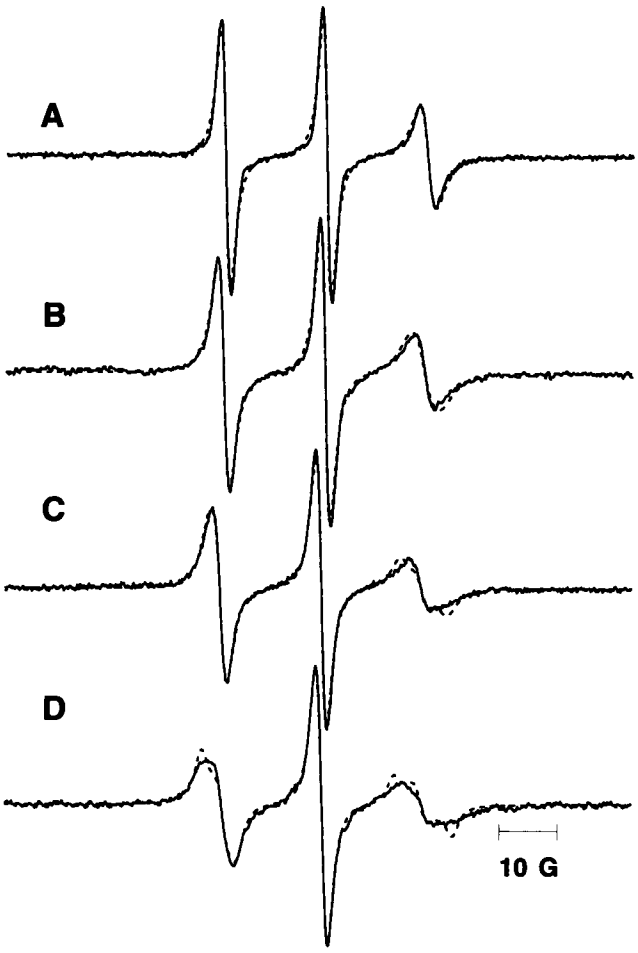

FIGURE 3 (*A*) 4-DUAP *Eco*RI 15mer (γ^2 = 14.1), (*B*) 9-DUAP *Eco*RI 26mer (γ^2 = 3.1), (*C*) 9-DUAP *Eco*RI 52mer (χ^2 = 10.2), and (*D*) (dT, DUAP)$_n$·(dA)$_n$ (χ^2 = 15.6) simulated according to the dynamic cylinder model. χ^2 Values were obtained from the spectral fitting program (Hustedt et al., 1993b).

flexible tethers (2–11 atoms long) to synthesize spin labels that monitor nucleic acid dynamics while minimizing any perturbation.

Although a tether that rigidly couples the nitroxide to motions of the base plane may seem to be an ideal probe, the attachment of a propyne group to the C5 position of a pyrimidine base has been found to stabilize the DNA helix (Froehler et al., 1992; Wagner et al., 1996). This may be the reason that monoacetylenic tethers have resulted in near rigid-limit EPR spectra. An example of this can be seen in the case of a monoacetylene-labeled 48mer used to calculate the persistence length of DNA (Hustedt et al., 1993a, 1995). The line-shape change observed between the rigid-limit spectrum and a DEAE-Sephadex-bound oligomer spectrum where the global dynamics are quenched but the internal dynamics are present is very small. The difference in the hyperfine A_{zz} tensor element is 1.03 G, and this value is considered to be proportional to the mean-squared amplitude from which the persistence length is calculated. If the elements of the hyperfine tensor change in value only as the result of a change in dynamics, then the trace will be constant, as a second-order tensor is rotationally invariant. Variance in the tensor trace is an indication that the tensor elements have changed as a result of polarity effects. When the monoacetylene-labeled 48mer rigid-limit tensor is compared with the bound oligomer tensor, the difference in 1/3 Tr = 0.25 G. This is a relatively small change, but this amount of variance is of the same order of magnitude as the difference in A_{zz} due to internal motions, which leads to an uncertainty in the persistence length of several hundred angstroms. If the monoacetylenic spin label is stabilizing the duplex as the near rigid-limit spectra seem to suggest, then it is doubtful that changes in internal dynamics of a duplex such as those associated with a helix conformational change can be observed.

In this paper, EPR spectra of DNA labeled with more flexible tethered labels of five atoms in length are presented, which show that both the global and internal dynamics of DNA are monitored. The experimental data obtained with the DUAP-labeled 15mer, 26mer, 52mer, and polymer demonstrates that five-atom-tethered spin labels are sensitive to changes in global diffusion. Although DUAP contains a more flexible tether than DUMTA, both spin labels are coupled to DNA dynamics. When the DUAP spectra are simulated according to the dynamic cylinder model (Table 1), the global diffusion is well modeled by the hydrodynamic cylinder equations. For a long helix such as the polymer, the global diffusion is in the rigid limit. The local order parameter S is found to be independent of helix length, consistent with the results obtained with DUMTA.

The DCAT-labeled duplexes in the B and Z forms show that five-atom-tethered labels are also sensitive to conformational changes. Inasmuch as the B-Z experiments were performed on polymers, the observed dynamic changes are internal. Table 5 lists the values of S for 5- and 11- (Strobel et al., 1995) atom-tethered spin labels incorporated into alternating copolymers and a plasmid. Although the change in S due to the B-Z transition for the 11-atom-tethered systems is small compared to the change observed with DCAT, the increase in ordering is consistently observed and reflects the small value of S due to the increased flexibility of this long tether. In Z-form DNA, the major groove is not as deep as in the B-form structure (see Fig. 3 of Strobel et al., 1990a). The C5 position of a Z-DNA cytidine is thus closer to the surface of the helix, placing the tethered nitroxide further into the surrounding solvent. If the nitroxide were not coupled to base motion, then the location of the spin label in Z-DNA should result in increased mobility of the probe. However, this is not what is experimentally observed. In Z-DNA, the spin label experiences both a decreased rate and an increased ordering compared with B-DNA. A control experiment where the EPR spectrum of (dA-dT, DUAT)$_n$ was measured in both low and high salt resulted in no change in the spectral line shape, indicating that the observed effect is due to neither a salt nor viscosity effect (Strobel et al., 1990a).

TABLE 1 Dynamic cylinder simulation parameters

Compound	g'_{xx}	g'_{yy}	g'_{zz}	A'_{xx} (G)	A'_{yy} (G)	A'_{zz} (G)	$T_{\parallel}$ (ns)	$T_{\perp}$ (ns)	θ_{dc} (°)	B* (G)
$(dT)_7DUMTA(dT)_7 \cdot (dA)_{15}$	2.0080	2.0060	2.0049	11.5	15.8	23.1	4.7	11.4	49	1.28
$[(dT)_7DUMTA(dT)_7]_2 \cdot (dA)_{30}$	2.0079	2.0061	2.0049	11.4	16.1	22.9	8.5	46.7	51	1.34
$[(dT)_7DUMTA(dT)_7]_3 \cdot (dA)_{45}$	2.0077	2.0063	2.0049	11.8	16.1	23.1	12.3	118	53	1.53
$[(dT)_7DUMTA(dT)_7]_m \cdot (dA)_n$	2.0077	2.0061	2.0051	11.6	16.7	23.1	RL#	RL	n/a	1.88
4-DUAP *Eco*RI 15mer	2.0071	2.0060	2.0055	12.2	15.9	20.0	4.7	11.4	49	1.01
9-DUAP *Eco*RI 26mer	2.0069	2.0061	2.0056	12.9	15.0	20.3	7.5	34.2	51	1.14
9-DUAP *Eco*RI 52mer	2.0069	2.0061	2.0056	12.9	15.0	20.2	14.0	166	50	1.08
$(dT, DUAP)_n \cdot (dA)_n$	2.0068	2.0062	2.0056	12.5	15.8	20.1	RL	RL	n/a	1.27
$(dG\text{-}dC, DCAT)_n$ B-DNA	2.0072	2.0064	2.0056	14.2	17.1	20.2	RL	RL	n/a	1.41
$(dG\text{-}dC, DCAT)_n$ Z-DNA	2.0074	2.0064	2.0054	12.2	16.5	22.1	RL	RL	n/a	1.62

*line broadening.
#Rigid limit.

TABLE 2 Base disk simulation parameters

Compound	g_{xx}	g_{yy}	g_{zz}	A_{xx} (G)	A_{yy} (G)	A_{zz} (G)	$\tau_{\parallel}$ (ns)	$\tau_{\perp}$ (ns)	θ_{bd} (°)	B (G)
$(dT)_7DUMTA(dT)_7 \cdot (dA)_{15}$	2.0096	2.0067	2.0028	7.47	7.21	36.3	0.50	1.4	40	0.80
$[(dT)_7DUMTA(dT)_7]_2 \cdot (dA)_{30}$	2.0096	2.0067	2.0028	7.47	7.21	36.3	0.50	2.4	40	0.80
$[(dT)_7DUMTA(dT)_7]_3 \cdot (dA)_{45}$	2.0096	2.0067	2.0028	7.47	7.21	36.3	0.50	3.6	40	0.80
$[(dT)_7DUMTA(dT)_7]_m \cdot (dA)_n$	2.0096	2.0067	2.0028	7.47	7.21	36.3	0.50	6.2	40	0.80
4-DUAP *Eco*RI 15mer	2.0090	2.0066	2.0029	6.40	5.90	36.2	0.10	1.5	40	0.60
9-DUAP *Eco*RI 26mer	2.0090	2.0066	2.0029	6.40	5.90	36.2	0.10	2.5	40	0.60
9-DUAP *Eco*RI 52mer	2.0090	2.0066	2.0029	6.40	5.90	36.2	0.10	4.6	40	0.60
$(dT, DUAP)_n \cdot (dA)_n$	2.0090	2.0066	2.0029	6.40	5.90	36.2	0.10	7.5	40	0.60
$(dG\text{-}dC, DCAT)_n$ B-DNA	2.0096	2.0067	2.0028	7.47	7.49	36.9	0.20	3.0	40	0.80
$(dG\text{-}dC, DCAT)_n$ Z-DNA	2.0096	2.0067	2.0028	7.47	7.49	36.9	0.20	9.7	40	0.80

TABLE 3 Rotational invariance of dynamic cylinder magnetic tensors

Compound	$\tilde{g}$	1/3Tr **g**	1/3Tr **g'**	$\bar{A}$ (G)	1/3Tr **A** (G)	1/3Tr **A'** (G)
$(dT)_7DUMTA(dT)_7 \cdot (dA)_{15}$	2.0063	2.0064	2.0063	16.9	17.0	16.8
$[(dT)_7DUMTA(dT)_7]_2 \cdot (dA)_{30}$	2.0063	2.0064	2.0063	16.9	17.0	16.8
$[(dT)_7DUMTA(dT)_7]_3 \cdot (dA)_{45}$	2.0063	2.0064	2.0063	16.9	17.0	17.0
$[(dT)_7DUMTA(dT)_7]_m \cdot (dA)_n$	2.0063	2.0064	2.0063	16.9	17.0	17.1
4-DUAP *Eco*RI 15mer	2.0062	2.0062	2.0062	16.1	16.0	16.0
9-DUAP *Eco*RI 26mer	2.0062	2.0062	2.0062	16.1	16.0	16.1
9-DUAP *Eco*RI 52mer	2.0062	2.0062	2.0062	16.1	16.0	16.0
$(dT, DUAP)_n \cdot (dA)_n$	2.0062	2.0062	2.0062	16.1	16.0	16.1
$(dG\text{-}dC, DCAT)_n$ B-DNA	2.0064	2.0064	2.0064	17.0	17.3	17.2
$(dG\text{-}dC, DCAT)_n$ Z-DNA	2.0064	2.0064	2.0064	17.0	17.3	16.9

In the base disk model, global tumbling, bending, and twisting are all subsumed into $\tau_{\perp}$ along with base dynamics (Keyes and Bobst, 1995). The value of $\tau_{\perp}$ increases with helix length due to its sensitivity to global diffusion. Comparison of $\tau_{\perp}$ for the DUMTA 30mer and DUAP 26mer yields a value of 2.4–2.5 ns, indicating good consistency between the two different tether lengths in detecting the dynamics of similar duplexes. Likewise, the DUMTA 45mer and DUAP 52mer display a $\tau_{\perp}$ between 3.6 and 4.6 ns, showing good correlation between the two labels. Since the global tumbling of a polymer will be in the rigid limit, $\tau_{\perp}$ will not be augmented by global diffusion and will reflect the internal dynamics alone. A value of several nanoseconds for $\tau_{\perp}$ has consistently provided good simulations for spin-labeled B-DNA polymers in the Bobst laboratory (Kao et al., 1983; Kao and Bobst, 1985; Pauly et al., 1987; Strobel et al., 1990b; Keyes and Bobst, 1995). The fact that spin labels with tethers of either two or five atoms in length reliably report nucleic acid dynamics nullifies the suggestion that these labels only monitor nitroxide motion independent of the base (Spaltenstein et al., 1989b).

Although studies with these spin labels have argued that the nitroxide motion is coupled to the base motion, no claim has been made regarding the absolute amplitude of base motion. It is expected that the dynamics of both nitroxide and base are not simple and that calculations of the amplitude of motion will depend strongly upon the geometric model used. Furthermore, due to the presence of the spin-label tether, a calculated value for the spin-label amplitude will probably be larger than the actual base amplitude. If an axially symmetric model is used to interpret the spin-label order parameter, then an angle determined by the relation

TABLE 4 Dynamic cylinder model results

Compound	τ_{rb} (ns)	S_g	S	S_z	$S^2\tau_{rb}$ (ns)
$(dT)_7DUMTA(dT)_7 \cdot (dA)_{15}$	8.5	0.39	0.33	0.33	0.93
$[(dT)_7DUMTA(dT)_7]_2 \cdot (dA)_{30}$	22.8	0.39	0.32	0.32	2.33
$[(dT)_7DUMTA(dT)_7]_3 \cdot (dA)_{45}$	39.1	0.39	0.32	0.32	4.00
$[(dT)_7DUMTA(dT)_7]_m \cdot (dA)_n$	n/a	0.34	0.31	0.31	n/a
4-DUAP *Eco*RI 15mer	8.5	0.21	0.20	0.20	0.34
9-DUAP *Eco*RI 26mer	18.5	0.18	0.21	0.21	0.82
9-DUAP *Eco*RI 52mer	50.7	0.18	0.21	0.21	2.24
$(dT, DUAP)_n \cdot (dA)_n$	n/a	0.18	0.20	0.20	n/a
$(dG\text{-}dC, DCAT)_n$ B-DNA	n/a	0.22	0.15	0.15	n/a
$(dG\text{-}dC, DCAT)_n$ Z-DNA	n/a	0.28	0.26	0.26	n/a

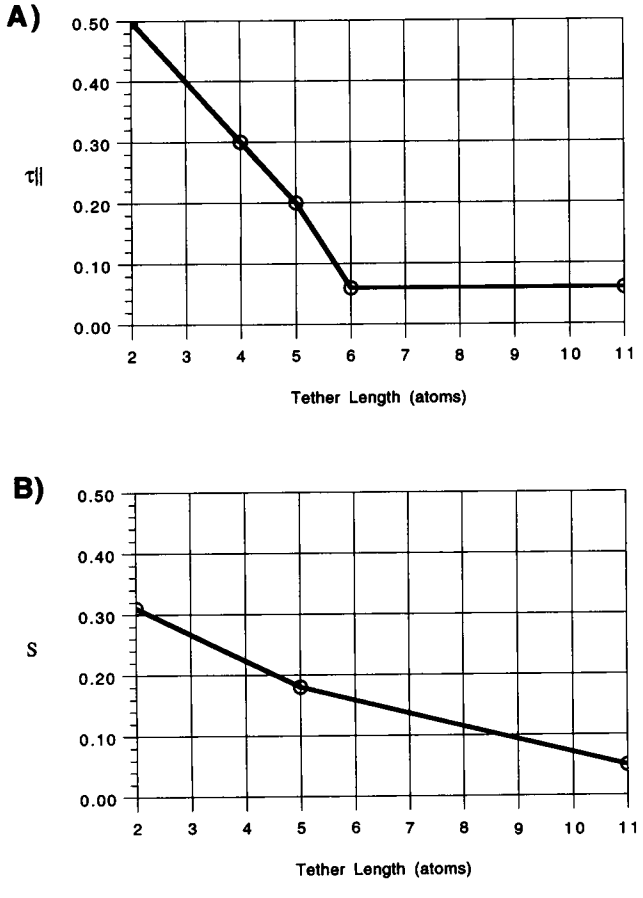

FIGURE 4 Effect of tether length on nitroxide motional (*A*) rate and (*B*) restriction. *Panel A* illustrates the trend in $\tau_{\|}$ for six-membered ring spin labels. Values are included for 2-atom (Keyes and Bobst, 1995), 4-atom (Bobst et al., 1984), 5-atom (Table 2), 6-atom (Bobst et al., 1984), and 11-atom (Strobel et al., 1990b) tethers. When the nitroxide is placed beyond the major groove, the value of $\tau_{\|}$ does not seem to change. *Panel B* displays the data for the order parameter S illustrating greater motional freedom for longer tethers attached to B-DNA. The value of S for the five-atom-tether is an average of the values for DUAP (0.20 ± 0.01) (Table 4), DCAT (0.15 ± 0.01) (Table 4), and DUAT (0.18 ± 0.01) (Keyes, 1994). The 11-atom tether S is an average of the values listed in Table 5.

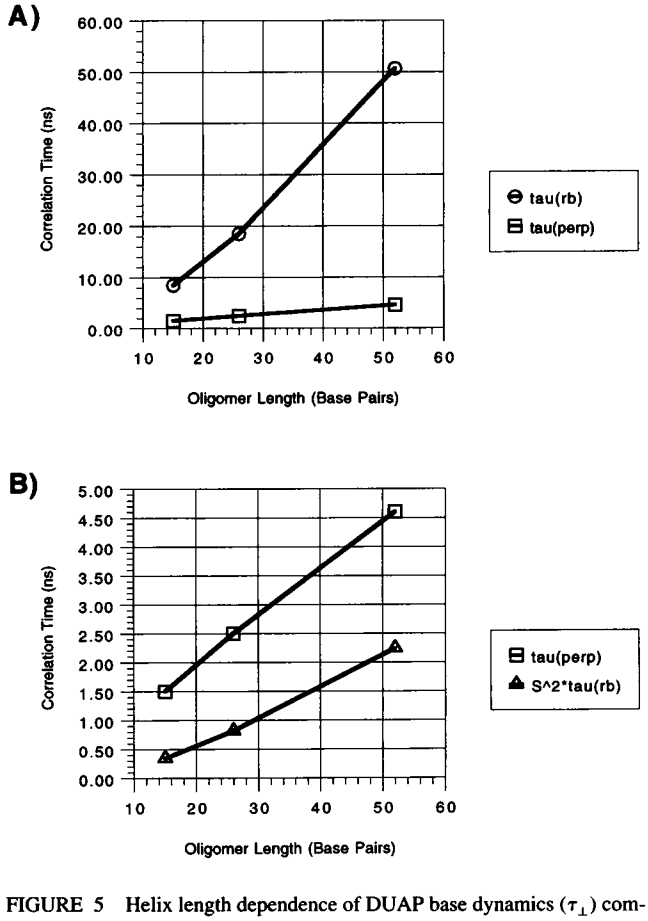

FIGURE 5 Helix length dependence of DUAP base dynamics ($\tau_{\perp}$) compared to (*A*) global tumbling (τ_{rb}) and (*B*) the component of global tumbling coupled to base dynamics through the order parameter ($S^2\tau_{rb}$).

$S = 0.5(3\cos^2\theta - 1)$ only describes the spin-label motion and does not directly provide the magnitude of base motion. What the order parameter in these studies does show is that the amplitude of base motion is linked to the conformation of the DNA. One instance where this has been found to be the case is in the B-Z transition, which correlates with a change in DNA rigidity (Strobel et al., 1995).

The order parameter S (Table 4) reflects changes in both $\tau_{\|}$ and $\tau_{\perp}$. Both S and $\tau_{\|}$ decrease as tether length increases (Fig. 4). When $\tau_{\perp}$ responds to a change in conformation, S changes as well. For instance, an increase in the value of S upon conversion of the B- to the Z-form of DNA corresponds to an increase in $\tau_{\perp}$ for the Z conformation. However, S is not dependent on the helix length. This implies that S is sensitive to the fraction of spin label dynamics

TABLE 5 Order parameters for B-Z conformational transition

Compound	B-DNA	Z-DNA
$(dG\text{-}dC, DCAT)_n$	0.15	0.26
$(dG\text{-}dC, DCAVAT)_n$	0.050	0.058
$(dG\text{-}dC, DCAVAP)_n$	0.050	0.077
(pRDZ8, DCAVAP)	0.053	0.068

reflecting internal base motion, but not to global motion, whereas $\tau_\perp$ is sensitive to both classes of motion.

Examination of Fig. 5 confirms that there is a strong correlation between $\tau_\perp$ and the helix length weighted by the square of the order parameter ($S^2\tau_{rb}$). This base-helix correlation concept yields an empirical relationship between spin-labeled base rate and amplitude. To complement this understanding, another approach to motional analysis is being pursued in parallel based upon the slowly relaxing local structure (SRLS) model (Polnaszek and Freed, 1975; Freed, 1977; Zager and Freed, 1982; Polimeno and Freed, 1995). The SRLS model contains similar parameters to those of the base-helix correlation concept as well as providing an internal correlation time, and accomplishes this within a single analysis. The internal dynamics are characterized by an order parameter and a correlation time describing a spin label rapidly diffusing within a local structure that relaxes on a longer time scale. Both the dynamic cylinder and base disk models are special cases of the SRLS model.

Conclusion

Analysis of the EPR spectra of DUAP-labeled nucleic acids corroborates the results obtained with DUMTA-labeled DNA (Keyes and Bobst, 1995), confirming the ability to effectively monitor both local and global dynamics with spin labels of different tether length. Examination of the B-Z transition detected by DCAT-labeled polymers extends previous work (Strobel et al., 1990b), demonstrating that five-atom-tethered nitroxides are sensitive to conformational changes and associated spin-labeled base ordering. By comparing the results obtained in this laboratory of several different spin-labeled systems, a unified understanding of local and global DNA dynamics has emerged.

For B-DNA, the dynamic cylinder model yields values of $S = 0.32 \pm 0.01$ (DUMTA), $S = 0.20 \pm 0.01$ (DUAP), and $S = 0.15 \pm 0.01$ (DCAT), while for DCAT-labeled Z-DNA, $S = 0.26 \pm 0.01$. These order parameters correspond to amplitudes of spin-labeled base motion that depend upon both tether length and DNA conformation. The fact that the amplitudes are conformation-dependent correlates with phenomena of biological interest such as DNA duplex rigidity and DNA bending. The global motion in the oligomer systems is effectively modeled as that of a hydrodynamic cylinder of a particular length. Application of the dynamic cylinder model should allow for monitoring changes in global dynamics such as those that occur with plasmid supercoiling and with protein binding.

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