A Probe for Sequence-Dependent Nucleic Acid Dynamics

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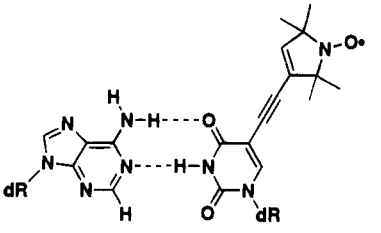
The internal dynamic properties of duplex DNA are complex, the time scales and amplitudes of these motions being the subject of current debate.[1] The existence of protein–DNA complexes in which DNA is dramatically bent and/or twisted relative to canonical double helices[2] raises interest in the energy cost of these distortions and motivates further study of DNA dynamics.[3] A central question is whether *sequence-dependent* differences in DNA flexibility, and hence dynamics, contribute to the selectivity with which DNA-deforming proteins bind to distinct DNA sequences.

We[1] and others[4] have previously monitored DNA dynamics using electron paramagnetic resonance (EPR) spectroscopy; we have favored spin-labeled base pair **1**. Although conveniently available,[5] this base pair is not optimal because the alkyne linkage does not fully constrain the nitroxide function to correlate its motion with that of the attached base. Consequently, EPR-based models of the motion of the nitroxide function cannot rigorously be held to reflect the dynamics of the nucleic acid itself. Of particular concern are motions we have observed on the sub-nanosecond time scale which could be attributed to either the base pair–probe ensemble, the probe itself, or some combination.[1] To address this limitation, we have synthesized phosphoramidite **5** and employed it in the synthesis of duplex DNA as base pair **2**, containing the 2-aminopurinyl (dP)[6] and quinolonyl (dQ) residues.[7,8] In B DNA, the highly constrained nitroxide-bearing ring is expected to project nondisruptively into the major groove.[7]

Phosphoramidite **5** was synthesized from the previously reported C-nucleoside **3**[8] as shown and was used to incorporate dQ centrally into DNA single strands.[9] That dQ had been incorporated was evidenced by quantitation of coupling yields (released trityl cation), the length of the strand as estimated by electrophoretic mobility (denaturing PAGE), the significant UV extinction coefficient in purified single strands at wavelengths



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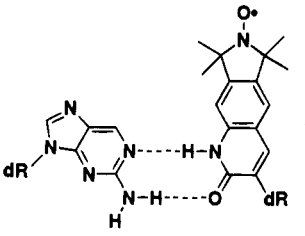
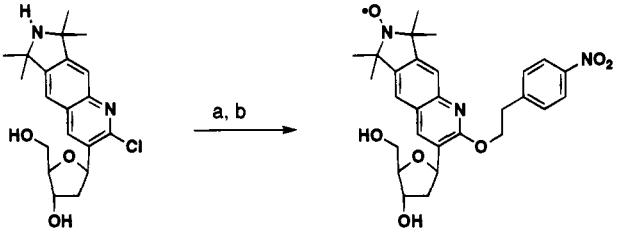


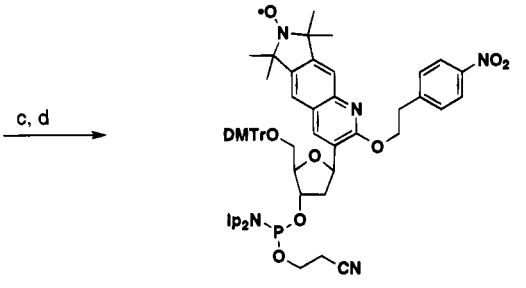
dP • dQ

2

greater than 300 nm where only dQ absorbs light, and the return of dQ (HPLC analysis) following digestion of the purified single strands with a phosphodiesterase/phosphatase mixture.[7,11]



3 **4**



5

a. $HOCH_2CH_2C_6H_4NH_2$, KH, THF (85%); b. MCPBA, CH_2Cl_2 (70%); c. 4,4'-DMTrCl, 4-DMAP, pyr (77%); d. $(Ip_2N)_2POCH_2CH_2CN$, $Ip_2NH_2^+ CHN_4^-$, CH_2Cl_2 (65%).

Native PAGE and UV-monitored thermal denaturation measurements confirmed the ability of several spin-labeled DNA strands to complex with partner strands. In a representative example, a 1:1 mixture of single strands **IB** and **IIB** (see Table 1) gave a predominant native PAGE band of lower mobility than the individual strands, but similar to the DNA duplex **IA·IIA** containing only the naturally occurring bases. Spin-labeled strand **IB** was admixed independently with each of the five potential partner strands **IIA**–**IIE**; for each pairing, the melting temperature and extent of hyperchromicity were quantified at 260 nm (Table 1). Consistent with the design, the highest melting spin-labeled duplex paired dQ to dP, as in **2**. All other pairings to **IB** were lower melting, including the pairings to dG (**IIC**) and dT (**IID**), both of which might have been expected to form wobble pairs containing two hydrogen bonds. In the

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(3) (a) von Hippel, P. H. *Science* **1994**, *263*, 769. (b) van der Vliet, P. C.; Verrijzer, C. P. *Bioessays* **1993**, *15*, 25.
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(6) The phosphoramidite necessary for synthesis of DNA containing dP is commercially available from Glen Research, Sterling, VA.
(7) Solomon, M. S.; Hopkins, P. B. *J. Org. Chem.* **1993**, *58*, 2232. Preliminary 2D ^{1}H NMR measurements following reduction of the nitroxide confirm the essential features of this structural model and will be reported elsewhere in due course.
(8) Miller, T. R.; Hopkins, P. B. *Bioorg. Med. Chem. Lett.* **1994**, *4*, 981.
(9) DNA was synthesized by the automated phosphoramidite method, except for incorporation of the dQ residue, which was conducted manually. Deprotection and cleavage from the resin were achieved by sequential treatment with DBU[10] and aqueous ammonia.
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(11) A cocktail of P1 nuclease, DNase I, snake venom phosphodiesterase, and calf intestinal alkaline phosphatase was used.

0002-7863/95/1517-9377$09.00/0

Table 1. Properties of Spin-Labeled DNA

5'CGAGGWCCAGC I 5'GACCPCATGYGAGC III

GCTCCXGGTCG5' II CTGGZGTACPCTGC5' IV

	W	X	Y	Z	T_m (°C)[a]	hyperchromicity (%)
IA·IIA	T	A			52	18
IB·IIB	Q	P			45	15
IB·IIC	Q	G			41	15
IB·IIA	Q	A			37	15
IB·IID	Q	T			36	12
IB·IIE	Q	C			31	11
IIIA·IVA			T	T	53	24
IIIA·IVB			T	Q	49	21
IIIB·IVA			Q	T	48	22
IIIB·IVB			Q	Q	43	20

[a] 6.8 μM in DNA strands, 10 mM phosphate (pH 7.0), brought to 115 mM Na^+ with NaCl, 0.1 mM EDTA.

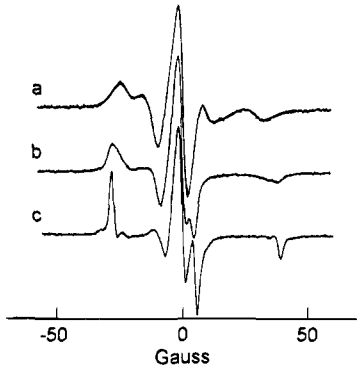

Figure 1. EPR spectra of (a) [5'-d(GCC TAC ATG QGA CG)·5'-d(CGT CPC ATG TAG GC)] (20 °C) overlaid with a simulated spectrum (see below); (b) [5'-d(GCC TAC ATG T*GA CG)·5'-d(CGT CAC ATG TAG GC)] (0 °C) in 50% w/v sucrose, where T* is in the spin probe as in **1**; (c) [5'-d(GCC TAC ATG QGA CG)·5'-d(CGT CPC ATG TAG GC)] (0 °C) in 50% w/v sucrose. Samples contained 10 mM phosphate buffer (pH 7.0), 115 mM Na^+, and 0.1 mM EDTA. A least-squares fit of the spectrum c following 1 G Lorentzian convolution yielded the following tensor values: A_{xx} = 6.68 G, A_{yy} = 5.41 G, A_{zz} = 33.9 G, g_{xx} = 2.0076, g_{yy} = 2.0061, g_{zz} = 2.0028 (χ^2 = 1.7).[13] The tensors were used to simulate spectrum a, yielding a single correlation time of 7.6 ns (χ^2 = 3.2) assuming an isotropic model.

several sequence contexts we have thus far studied, duplexes containing the base pair **2** were lower melting than analogues containing a dA–dT or a dP–dT pair, an effect which was amplified in doubly spin labeled duplexes (see **III·IV**). The reasons for this relative destabilization of the duplex are at present unknown.

The circular dichroism (CD) spectrum of the spin-labeled DNA **IB·IIB** possessed negative and positive molar ellipticities at ca. 250 and 280 nm, respectively, characteristic of right-handed double helices. However, the molar ellipticity per residue from 225 to 300 nm was more negative than in the unlabeled DNA **IA·IIA**. Because the impact of the novel chromophores in **2** on the CD spectrum is unknown, no further structural conclusions have been drawn.

DNAs labeled with dQ have provided the first glimpse by EPR of nucleic acid dynamics uncompromised by independent spin probe motion. As with less rigidly tethered nitroxides, single strands and short and long DNA duplexes labeled as in **2** were easily distinguished from one another by their EPR spectra (data not shown).[12] Optimal simulations of the EPR spectra of DNA duplexes labeled as in **1** require the assumption of tensors preaveraged by low-amplitude, high-frequency motions.[1] In contrast, simulation[13] of a 14-mer duplex, labeled as in **2**, in solution at 20 °C employing *typical rigid limit tensors* afforded a very good fit (Figure 1a).[14,15] The small hydrodynamic anisotropy of the 14-mer (ca. 2) suggests that a model assuming isotropic rotational motion is reasonable.[1] The best fit correlation time (7.6 ns, χ^2 = 3.2) agrees quite well with that predicted by hydrodynamic theory (7.0 ns).[16] This demonstrated that global tumbling, rather than sub-nanosecond internal motion, dominates the decorrelation process of probe **2**. Further indication of the difference between labels **1** and **2** is found with DNA duplexes immobilized (on the EPR time scale) in aqueous sucrose, where the narrower line widths of spectra from **2** (Figure 1c) relative to **1** (Figure 1b) are indicative of highly constrained dynamics. Thus, **2** achieved the goal of rigorous correlation of the dynamics of the biopolymer and label.

Spin-labeled base pair **2** thus appears to be accommodated in duplex DNA and rigorously to correlate motion of the spin label with the attached base. Without the latter feature, probe motion independent of the macromolecule is indistinguishable from local, length-independent motions of the macromolecule itself. Studies with **2** will not suffer this fundamental limitation which has plagued previous studies. Further studies will be reported in due course.

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Supporting Information Available: Synthesis procedures and spectroscopic properties of **5** and synthetic intermediates, DNA synthesis procedures, UV-monitored thermal denaturation curves, CD spectra, and EPR spectra (7 pages). This material is contained in many libraries on microfiche, immediately follows this article in the microfilm version of the journal, can be ordered from the ACS, and can be downloaded from the Internet; see any current masthead page for ordering information and Internet access instructions.

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(15) Tensors were obtained from a least-squares fit[13] of a rigid spectrum (14-mer duplex immobilized in aqueous sucrose) convolved with a Lorentzian of 1 G (see Figure 1 legend). The convolution was necessary because the rigid spectrum, which was narrower than expected (0.5 vs 3.0 G line width), displayed ^{13}C satellite peaks which interfered with the successful completion of the least-squares fit.

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