

A Solid-State Deuterium NMR Study of Furanose Ring Dynamics in $[d(\text{CGCGAATTCGCG})]_2$

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Abstract: Solid-state deuterium NMR spectroscopy has been used to characterize the dynamics of the furanose rings of A5 and A6 (and by symmetry A17 and A18) in the self-complementary DNA dodecamer duplex $[d(\text{CGCGAATTCGCG})]_2$, which contains the *EcoRI* binding site. 2'-Deoxyadenosine, deuterated at the 2'' position by direct chemical methods, was incorporated into the A5 and A6 sites of the dodecamer by using the solid-phase phosphite triester method. ²H NMR line shape studies of monomeric 2'-deoxyadenosine-2''-d₁ yielded a QCC_{eff} of 172 kHz. Assuming a $\text{QCC}_{\text{static}}$ of 178 kHz, as derived from NQR studies of tetradeuteriofuran, and a static asymmetry parameter of zero, this line shape may be simulated by assuming a two-site libration of the furanose ring with an amplitude of $\pm 9^\circ$. Relaxation studies of the selectively deuterated DNA dodecamer indicate that at high levels of hydration, the T_1 of the 2'' deuterons varies in constant proportion to the T_1 of the base deuterons, implying that the dynamics of the furanose rings and the bases may be coupled. Simulation of ²H NMR line shapes over a hydration range varying from $W = 0$ to $W = 28$ (mol H₂O/mol nucleotide) shows a small decrease in QCC_{eff} from 172 to 146 kHz, which precludes the occurrence of large amplitude ($\gg 20^\circ$) dynamics in these furanose rings over this hydration range. Line shape perturbations produced by intermediate regime motions may be simulated by using models of restricted reorientation about the helix axis, increasing in rate and amplitude at higher hydration levels. DNA samples equilibrated at 92% relative humidity produce line shapes consistent with the formation of a liquid crystal phase.

Introduction

The internal dynamics of polynucleotides continues to be the subject of numerous theoretical and experimental investigations, with considerable controversy persisting over the amplitude, rate, and degree of localization of internal motions in DNA. Although high-resolution NMR relaxation studies appear unanimous in the observation of large amplitude, localized motions of the base and deoxyribose moieties at rates greater than 10^8 Hz,^{1,2} FPA and EPR studies indicate that internal motions in DNA may be the result of coupled torsions and bendings of the DNA helix.³⁻⁵ It is important to settle the question of the nature of internal motions in polynucleotides, both for the insight that such motions may give in understanding the biological function of these molecules, and to understand the allowed range of deviation from defined structures.

Concerning localized motions of the furanose rings in polynucleotides, primary sources of experimental information include high-resolution NMR relaxation parameters, averaged scalar coupling constants, and X-ray diffraction data. Furanose rings in nucleosides are asymmetrically substituted, and therefore the conformations assumed by the ring are not evenly weighted on the pseudorotation cycle. In fact, X-ray crystallographic studies of polynucleotides indicate that the conformations assumed by the sugar ring tend to cluster at two distinct and relatively narrow puckering conformations, namely C2'-endo and C3'-endo.⁶⁻⁸

The relative conformational energies of the C2'-endo and C3'-endo puckering modes have been the subject of several theoretical studies. For example, Levitt and Warshel⁹ utilized a potential energy analysis, where the energy parameter was obtained through analysis of a large body of X-ray crystallographic, calorimetric, and spectroscopic data, and concluded that the energy barrier for the sugar conformation interconverting between C2'-endo and C3'-endo was only 1.4 kcal/mol. Olson and co-workers carried out a series of statistical computations to test various theoretical potential energy estimates of furanose pseudorotation¹⁰ and suggested that the presence of endocyclic and exocyclic substituents in the sugar moiety introduces a potential barrier that opposes free pseudorotation.

NMR relaxation studies of internal dynamics in high molecular weight DNA have largely supported the occurrence of substantial and localized motions of the furanose rings on the time scale of

nanoseconds. For example, on the basis of high-resolution ³¹P and ¹³C relaxation measurements, Jardetzky and co-workers¹ reported ³¹P-¹H vector fluctuations of $\pm 27^\circ$ with a time constant of 2.2×10^{-9} s, base plane fluctuations of $\pm 20^\circ$ with a time constant of 10^{-9} s, and fluctuations of the deoxyribose ring of $\pm 20^\circ$ to $\pm 33^\circ$ with a similar time constant. A similar view of the internal dynamics of very long DNA fragments has been reported in a series of papers by James and co-workers.¹¹

Clore and Gronenborn¹² have reported a study of furanose ring dynamics in synthetic oligonucleotides. In that work, cross relaxation rates between proton pairs were determined from the initial slope of the NOE buildup curves, from which the apparent correlation times were calculated by using a model of internal motion which included a two-site jump and restricted diffusion or wobble within a cone, with the whole molecule assumed to undergo an isotropic tumbling motion. The results were claimed to indicate that the correlation time of the H2'-H2'' vector fluctuation is 3-fold shorter than that of the H5-H6 vector and of the order of 10^{-9} s.

By far the most exhaustive study of sugar ring conformational dynamics has been performed by Altona and co-workers who, drawing from a large body of X-ray crystallographic data and ¹H NMR coupling constants, constructed a set of formulas which relate the magnitude of the scalar coupling constant between a given proton pair to the relevant bond torsional angles.¹³⁻¹⁷ In

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this empirical approach, endocyclic torsional angles in five-membered rings were described by a pseudorotation phase angle and an amplitude of puckering, and the authors suggested that conformational interconversion is a process of hindered pseudorotation with a correlation time on the nanosecond time scale, proceeding through the O4'-endo conformation and maintaining a constant puckering amplitude of $\sim 38^\circ$.

Bax and Lerner¹⁸ have analyzed the H2'-H2'' and H3'-H4' coupling constants of the sequence [d(CGCGAATTCGCG)]₂ utilizing a program developed by Altona and co-workers. By confining the minor conformer to the 3'-endo conformation with a 36° amplitude of pucker, the analysis indicated that the population of the major conformer ranges from 74% to 99% with a puckering amplitude of $33^\circ \sim 38^\circ$ for the individual deoxyribose sugar moieties in the sequence.

A somewhat different view of the structure of the DNA dodecamer [d(CGCGAATTCGCG)]₂ has been reported by Reid and co-workers.¹⁹ That structure, derived by using distance geometry techniques, displays kinks in the C3-G4 base step and in the A6-T7 base step, which appear similar to those reported for the *EcoRI* restriction site DNA bound to its endonuclease.²⁰ Although this solution structure differs in several respects from the crystal structure as reported by Dickerson and Drew,²¹ the conformations reported by both studies for the sugar rings of A5 and A6 are quite similar, the NMR study reporting ¹T conformations for both sugars and the X-ray study reporting ₁E (C1'-exo) conformations for both sugars.

It seems inevitable that controversy will persist over the nature of furanose ring structure and dynamics because the interpretation of high-resolution NMR relaxation data requires numerous assumptions concerning the relaxation mechanism and the structure, while investigations of DNA structure by two-dimensional NMR methods require assumptions about the dynamics; the problems are completely coupled. Solid-state deuterium NMR spectroscopy, however, is capable of providing completely independent information on the rates and amplitudes of molecular dynamics.²² The utility of ²H NMR lies in the fact that both the relaxation of deuterium and the ²H-NMR line shapes are dominated by interactions between the electric quadrupole of the ²H nucleus and surrounding electric field gradients. Therefore, ²H-NMR line shapes and relaxation can be treated quantitatively without the necessity of assuming a detailed molecular structure. In addition, ²H NMR is capable of probing a wide dynamic range of motions, in that the combination of spin alignment techniques, quadrupolar echo line shape analysis, and *T*₁ relaxation data together allow the investigation of motional rates spanning almost ten orders of magnitude.

In recent years, numerous ²H-NMR studies of solid samples of polynucleotides have been published.²³⁻²⁶ These studies have been exclusively confined to the dynamics of purine bases in samples of hydrated, high molecular weight DNAs, in which the purine H8 position has been deuterated by exchange in D₂O at pD = 7. In addition, we have recently reported a ²H-NMR study of a DNA dodecamer,²⁷ selectively deuterated on specific pyri-

midine bases, and a second study of a methyl-deuterated dodecamer has been recently completed.²⁸ Although ²H-NMR studies have done much to advance our knowledge about the motion of bases in polynucleotides, the method has yet to be extended to other structural features such as the furanose rings or the phosphodiester backbone.

Torchia and co-workers^{29,30} have reported a solid-state ²H-NMR investigation of sugar ring flexibility in single nucleosides, where an enzymatic synthesis was used to produce 2'-deoxyguanosine-2'-*d*₂ and 2'-deoxythymidine-2'-*d*₂ (both protons at the C2' ring position were deuterated). This investigation showed significantly shorter *T*₁ values than expected for a rigid system: <1 s for deoxyguanosine and ~ 0.15 s for deoxythymidine. The authors suggested the presence of small amplitude motion of the furanose ring on a time scale of $<10^{-6}$ s which may be independent of the nature of the base. The authors also observed only about one-half of the expected ²H signal intensity of deoxyguanosine when compared with a known weight of methylene-deuterated polyethylene and interpreted this fact as indicating that a significant fraction of the sample undergoes motion of the furanose ring on a time scale within the intermediate rate regime. The authors suggested that this may be due to interconversion between different sugar ring conformations.

In this paper we report the first solid-state deuterium NMR study of furanose ring dynamics in the self-complementary DNA dodecamer [d(CGCGAATTCGCG)]₂. This dodecamer, which contains the *EcoRI* endonuclease restriction site d(GAATTC), was selectively deuterated at the H2'' position on the furanose rings of A5 and A6 (and by symmetry A17 and A18). The synthesis of [d(CGCGA*A*TTTCGCG)]₂ will first be described. As mentioned above, it is possible to deuterate both the H2' and the H2'' positions by enzymatic methods, but our approach involved the production of selectively deuterated deoxyadenosine by direct chemical synthesis. This approach has the advantage of being stereospecific as well as very high in yield. Next we will describe a ²H-NMR study of monomeric 2''-deoxyadenosine-2''-*d*₁ which was performed for the purpose of characterizing the static electric field gradient (EFG) tensor of the 2'' deuteron. Finally, a ²H-NMR line shape study of the selectively deuterated DNA duplex will be presented, with the goal of deriving a model that describes the amplitude and rate of the motion of the furanose ring as a function of hydration level as well as accounting for whole-molecule motions in a way consistent with our studies of base-deuterated DNA dodecamers.^{27,28}

Experimental Section

Materials and Methods. Labeled Compounds. 1,3-Dichloro-1,1,3,3-tetraisopropylsiloxane (TPDSCI₂), phenyl chlorothionformate, 4-(*N,N*-dimethylamino)pyridine (DMAP), tetra-*n*-butylammonium fluoride (TBAF), 4,4'-dimethoxytrityl chloride, trimethylchlorosilane, and benzoyl chloride were purchased from Aldrich Chemical Company. 2,2'-Azobis(2-methylpropionitrile) (AIBN) was purchased from Kodak and tri-*n*-butyltin deuteride was purchased from Strem Chemicals. Adenosine, 2'-deoxyadenosine, and 5'-*O*-(dimethoxytrityl)-6-*N*-benzoyl-2'-deoxyadenosine were purchased from Sigma. Pyridine and dichloromethane were refluxed from calcium hydride for 2-4 h and then distilled under nitrogen before use in reactions. All reactions were carried out under an atmosphere of dry nitrogen.

Kieselgel 60 (230-400 mesh) from E.M. Science was used for column chromatography. Analytical TLC was carried out by using Kieselgel 60 F₂₅₄ glass backed plates (E.M. Science). Dowex 1-X2 resin (Aldrich) was used for anion exchange chromatography; the resin was washed with 0.5 M sodium hydroxide solution and then rinsed with water before use.

Solution ¹H NMR spectra were recorded on a Bruker WM-500 spectrometer. DNA was prepared on an Applied Biosystems Model 380A Synthesizer by using the solid phase phosphite triester method.

3',5'-O-(1,1,3,3-Tetraisopropyl-1,3-disiloxanediy)adenosine. This compound was prepared by using the method of Robins et al.³¹ TPD-

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SiCl₂ (2.36 g, 7.49 mmol) was added to a suspension of dried adenosine (2.00 g, 7.49 mmol) in dry pyridine (80 mL), and the mixture was stirred at room temperature for 3 h. The solvent was removed under vacuum, and the residue was partitioned between ethyl acetate and water. The organic phase was washed successively with cold aqueous 1 M HCl, water, aqueous saturated sodium bicarbonate solution, and aqueous saturated sodium chloride solution, then dried (sodium sulfate), and evaporated. The colorless residue contained minor contaminants which were removed by chromatography on a silica gel column (3 cm diameter × 22 cm length) using dichloromethane/methanol (92/8, v/v) to provide 3.25 g (85% yield) of 3',5'-protected adenosine.

2'-O-[(Phenoxythio)carbonyl]-3',5'-O-(1,1,3,3-tetraisopropyl-1,3-disiloxanedyl)adenosine. This compound was prepared by using the method of Robins et al. with some modification. 3',5'-O-TPDS-adenosine (2.80 g, 5.49 mmol) was dissolved in dichloromethane (70 mL) and then DMAP (1.376 g, 11.3 mmol) and phenyl chlorothionoformate (1.422 g, 8.24 mmol) were added. The mixture was stirred for 2 h at room temperature. The solvent was evaporated, and the residue was processed in the same manner as the preceding step to give a yellow glass. Silica gel chromatography (3 cm × 15 cm) of this material with use of dichloromethane and then dichloromethane/methanol (95/5, v/v) provided 3.40 g (96% yield) of 2'-O-[(phenoxythio)carbonyl]-3',5'-O-TPDS-adenosine as a pale yellow solid.

2'-Deuterio-2'-deoxy-3',5'-O-(1,1,3,3-tetraisopropyl-1,3-disiloxanedyl)adenosine. This compound was prepared by using the general method of Robins et al. The (phenoxythio)carbonyl ester (2.00 g, 3.10 mmol) dissolved in dry toluene (70 mL), and then AIBN (0.102 g, 0.62 mmol) and tri-*n*-butyltin deuteride (1.358 g, 4.66 mmol) were added. The solution was cooled to -78 °C and deoxygenated with five freeze-pump-thaw cycles and then heated at 95 °C (oil bath temperature) for 2 h. The solvent was evaporated, and the residue was subjected to chromatography (3 cm × 18 cm) by using dichloromethane and then dichloromethane/methanol (94/6, v/v) to provide 1.42 g (93% yield) of pure material as a colorless solid. The product obtained had identical chromatographic mobility with the TPDS derivative of 2'-deoxyadenosine.

2'-Deoxyadenosine-2''-d₁. The disiloxane (1.2 g, 2.43 mmol) was dissolved in THF (35 mL), and a 1.1 M solution of TBAF in THF (4.4 mL, 4.86 mmol) was added. The reaction mixture was stirred at room temperature for 30 min after which the solvent was evaporated. The residue was partitioned between diethyl ether and water, and the aqueous phase was concentrated and loaded onto a column of Dowex 1-X2 (hydroxide form). The 2''-deuterated adenosines were eluted with water, and the appropriate fractions were combined and lyophilized to provide 0.60 g (98% yield) of product. The deuterium-labeled product and authentic 2'-deoxyadenosine had identical chromatographic mobility: ¹H NMR (methanol-*d*₄) indicated enrichment at the 2'' site of about 90%; ¹H NMR (methanol-*d*₄) δ 8.34 (s, 1 H, H8), 8.21 (s, 1 H, H2), 6.46 (d, *J* = 7.5 Hz, H1'), 4.62 (dd, 1 H, *J* = 3.0 and 5.8 Hz, H3'), 4.11 (m, 1 H, H4'), 3.89 (dd, 1 H, *J* = 4.1 and 12 Hz, H5'), 3.78 (dd, 1 H, *J* = 4.1 and 12.0 Hz, H5''), 2.83 (apparent t, ~0.9 H, *J* = 7.5 Hz, H2'), 2.43 (br s, ~0.1 H, *W*_{1/2} ~ 10 Hz, H2'').

5'-O-(Dimethoxytrityl)-6-*N*-benzoyl-2'-deuterio-2'-deoxyadenosine. This compound was prepared by using the method of Ti et al.³² 2''-Deuterium-labeled adenosine (526 mg, 2.09 mmol) was dried by evaporation of pyridine (3 × 8 mL) and then suspended in 24 mL of dry pyridine. DMAP (20 mg, 0.16 mmol), triethylamine (296 mg, 2.92 mmol), and 4,4'-dinitroxytrityl chloride (880 mg, 2.60 mmol) were added, and the mixture was stirred at room temperature for 2.5 h. The mixture was cooled in an ice bath, and trimethylchlorosilane (1.134 g, 10.44 mmol) was added dropwise. After 15 min, benzoyl chloride (1.468 g, 10.44 mmol) was added (at 0 °C), and the mixture was stirred at room temperature for 3 h. The reaction mixture was cooled in an ice bath, and water (5.0 mL) was added. After 5 min, concentrated aqueous ammonia (5.0 mL) was added, and the mixture was stirred for 30 min at room temperature. The mixture was concentrated in vacuo, and the residue was partitioned between diethyl ether and water. The ether layer was dried (sodium sulfate), and the solvent was evaporated to provide an orange gum which was subjected to chromatography (2 cm × 22 cm). Elution with dichloromethane followed by dichloromethane/methanol (98/2, v/v) provided 822 mg (60% yield) of the product as a colorless solid. The deuterium-labeled derivative and the corresponding unlabeled compound had identical chromatographic mobility and near-identical ¹H NMR spectra: ¹H NMR (methanol-*d*₄) δ 6.56 (d, 1 H, *J* = 7.5 Hz, H1'), 4.72 (dd, 1 H, *J* = 3.0 and 6.0 Hz, H3'), 4.21 (m, 1 H, H4'), 3.39 (m, 2 H, H5' and H5''), 3.06 (apparent t, ~0.9 H, *J* = 7.5 Hz, H2'), 2.57 (br s, ~0.1 H, *W*_{1/2} ~ 10 Hz, H2'').

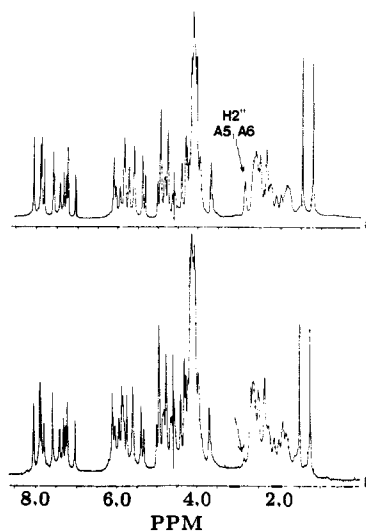


Figure 1. (a) High-resolution ¹H-NMR spectrum of the DNA dodecamer [d(CGCGAATTCGCG)]₂. Assignment of the ¹H spectrum by two-dimensional NMR techniques⁶¹ shows that the H2'' protons of A5 and A6 (and by symmetry also A17 and A18) are degenerate with a chemical shift of 2.88 ppm relative to DSS. (b) High-resolution ¹H-NMR spectrum of the same DNA dodecamer deuterated at the 2'' positions of A5 and A6 (also A17 and A18), indicating ²H enrichment of about 90%.

5'-O-(Dimethoxytrityl)-6-*N*-benzoyl-2'-deuterio-2'-deoxyadenosine 3'-(2-(Cianoethyl)-*N,N*-diisopropylamino)phosphoramidite. This compound was prepared by using the method of Barone et al.³³ The dimethoxytrityl derivative (750 mg, 1.14 mmol) and diisopropylammonium tetrazolidate (98 mg, 0.57 mmol) were dissolved in dichloromethane (4 mL) and bis(diisopropylamino)(cyanoethoxy)phosphine (411 mg, 1.37 mmol) was added dropwise. The reaction mixture was stirred at room temperature for 1 h, then diluted with dichloromethane (10 mL) and washed with cold saturated sodium bicarbonate solution (2 mL). The dichloromethane layer was dried (sodium sulfate), and the solvent was evaporated to give a yellow oil. Column chromatography (2 cm × 22 cm) using ethyl acetate afforded 628 mg (64% yield) of the deuterium-labeled phosphoramidite as a colorless oil which was freeze-dried from benzene to provide a colorless dry powder. This material possessed identical TLC mobility with the corresponding unlabeled adenosine phosphoramidite and very similar ¹H NMR spectral characteristics.

DNA Synthesis and Purification. The deuterium-labeled deoxy-dodecanucleotide was synthesized by the solid-phase phosphite triester method by using an Applied Biosystems (ABI) Model 380A Synthesizer. A standard 10-μmol program from ABI was used. After deprotection in concentrated ammonia (60 °C, 48 h) the solvent was evaporated, and the pellet precipitated from aqueous 1 M NaCl/ethanol (2 mL/8 mL) at -20 °C overnight. Centrifugation and removal of the supernatant provided a colorless precipitate which was dissolved in water (1 mL), loaded onto a Sephadex G-25 column (2.5 cm × 120 cm), and eluted with water. Fractions containing DNA were detected by absorbance at 260 nm, assayed for purity by polyacrylamide gel electrophoresis, and pooled appropriately. Lyophilization gave the 2''-deuterium-labeled deoxy-dodecanucleotide [d(CGCGA*A*TCGCG)]₂ as a colorless solid. Two 10-μmol syntheses were carried out to yield approximately 50 mg of purified, labeled DNA. Figure 1 shows a comparison of the high-resolution ¹H NMR spectra of labeled and unlabeled [d-(CGCGAATTCGCG)]₂. Assignment of the proton spectrum by two-dimensional NMR methods^{19a} indicates that the 2'' protons of A5 and A6 (and by symmetry A17 and A18) are degenerate with a chemical shift of 2.88 ppm relative to DSS. Inspection of Figure 1 indicates deuterium enrichment at the 2'' sites of A5 and A6 (also A17 and A18) of about 90%.

Prior to study by solid-state ²H NMR, to the lyophilized, desalted DNA was added 10% sodium chloride by weight. The samples were then dissolved in deuterium-depleted water and again lyophilized. The dry DNA was packed into a 5 mm × 15 mm glass tube and hydrated by vapor equilibration against saturated salt solutions (in deuterium-depleted H₂O) of known relative humidity.³⁴ Samples were allowed to equilibrate

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Table 1^a

		5'-CGCGA*A*TCGCG-3' 3'-GCGCTTA*A*GCGC-5'				
		static quadrupole coupling constant (QCC) = 178 kHz static asymmetry parameter (η) 0.00.				
relative humidity (RH)	dry	66%	80%	88%	92%	
W (H ₂ O/nucleotide)	0.0	4.1	10.4	15.3	28.3	
T_1 (s)	1.80	1.04	0.110	0.070	0.089	
T_2 (μ s)	330	330	190	150	112	
QCC _{eff} (kHz)/ η	172/0.04	170/0.05	161/0.06	155/0.05	146/0.04	
two-site libration	$\pm 9^\circ$	$\pm 10^\circ$	$\pm 13^\circ$	$\pm 14^\circ$	$\pm 16^\circ$	
biaxial libration ($\pm\theta, \pm\phi$)	$\pm 12^\circ, \pm 4^\circ$	$\pm 13^\circ, \pm 4^\circ$	$\pm 18^\circ, \pm 8^\circ$	$\pm 18^\circ, \pm 14^\circ$	$\pm 22^\circ, \pm 18^\circ$	
(rms)	8.9°	9.6°	13.9°	16.1°	20.1°	
cone diffusion (S_{zz}, θ_0)	0.97, 11.5°	0.96, 14.1°	0.90, 21.5°	0.87, 24.5°	0.82, 28.9°	

^a Motional amplitudes in Table 1 were calculated from a fit to QCC_{eff}. See text for details.

for 2–3 weeks to ensure complete and uniform hydration. Water adsorption as a function of humidity was monitored gravimetrically, a blank glass tare being used to determine the degree of water adsorption onto the glass sample tube. The difficulty in removing all the water from DNA, even with extensive pumping has been noted,⁶¹ but the error introduced by the assumption that the lyophilized samples were dry is small and will result in reported hydration levels being slightly reduced from real values. Assuming the weight of the lyophilized DNA is close to a dry weight, water absorption W (mol water/mol nucleotide) for each relative humidity can be determined, and the results are shown in Table 1.

Solid-State NMR Spectroscopy. Solid-state ²H-NMR spectra were obtained at 76.76 MHz on a home-built NMR spectrometer³⁵ by using an eight-step phase-cycled quadrupolar echo pulse sequence.³⁶ Ninety degree pulse times were typically less than 3 μ s. Data acquisition was initiated prior to the top of the quadrupolar echo, and the time domain data left shifted to the echo maximum prior to Fourier transformation, by using a fractional left shift algorithm with five point smoothing.³⁷ Lorentzian line broadening of 1500–2000 Hz was used to obtain adequate signal-to-noise. Simulations of ²H NMR line shapes were obtained by using the program MXQET as described by Greenfield et al.,³⁸ which can accommodate various motional models including multiaxis and multisite jumps. Spectra of aligned DNA phases were simulated by using the modified version GNMxQET.

Deuterium spin–lattice relaxation times (T_1) were determined by using a saturation-recovery pulse sequence, consisting of a comb of 90° pulses separated by short delays of $\tau \ll T_1$, and followed, after a suitable recovery period, by a quadrupolar echo sequence. The recovered magnetization was determined either from the height of the quadrupolar echo, resulting in a "powder-averaged" (T_1) measurement, or from the amplitude of the perpendicular edge of the powder pattern. When appropriate, correlation times τ_c were calculated by utilizing expressions for multisite jumps as described by Torchia and Szabo.³⁹ Transverse relaxation times (T_{2e}) were determined from echo heights by varying the 90° pulse spacing in the quadrupolar echo sequence.

In this paper the following conventions in notation will be observed. For an isolated deuteron in a solid NMR frequency is given by⁴⁰

$$\omega = \omega_0 \pm \omega_Q$$

where ω_0 is the Zeeman frequency and ω_Q is given by

$$\omega_Q = \frac{3\pi}{4} \frac{e^2qQ}{h} (3 \cos^2 \Theta - 1 - \eta \sin^2 \Theta \cos 2\Phi)$$

The quantity e^2qQ/h is designated the quadrupolar coupling constant (QCC), and the asymmetry parameter η describes the deviation of the electric field gradient (EFG) tensor from axial symmetry in the principal axis system (PAS). Θ and Φ are the polar angles locating the magnetic field vector B_0 in the PAS of the EFG tensor.

Throughout this paper motional rates will be assigned to three regimes: fast, intermediate, and slow. These regimes are measured relative to the time scale of the ²H NMR experiment which is roughly $1/\omega_Q$. Thus the

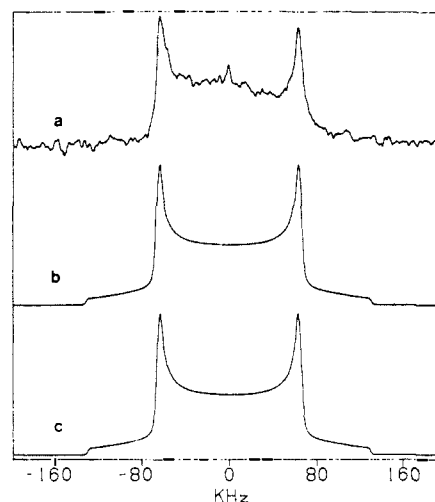


Figure 2. (a) Solid-state ²H-NMR spectrum of 2''-deuterio-2'-deoxyadenosine obtained at 76.76 MHz by using a quadrupole echo sequence (1100 scans, relaxation delay = 180 s). (b) Calculated spectrum based on a rigid lattice model yielding $\eta_{\text{eff}} = 0.02$ and QCC_{eff} = 172 kHz. (c) Calculated spectrum assuming a small amplitude libration ($\pm 8^\circ$) of the 2' methylc group.

fast motion limit corresponds to motions with correlation times $\tau_c \ll 1/\omega_Q$ which, for deuterium, corresponds to motional rates of over 10^7 Hz. In the fast motion limit, anisotropic averaging of the EFG tensor allows the simulation of powder patterns by using an effective coupling constant QCC_{eff} and an effective asymmetry parameter η_{eff} . Intermediate regime motions correspond to correlation times on the order of $1/\omega_Q$ or rates ranging from about 10^4 – 10^6 Hz. Slow motions correspond to $\tau_c \gg 1/\omega_Q$ or rates less than 10^4 Hz.

Results

Furanose Ring Dynamics in 2'-Deoxyadenosine-2''-d₁. (T_1) of the 2'' deuteron in deoxyadenosine was measured at 298 K by using a saturation/recovery pulse sequence, as described above, and varying the recovery time from 10 ms to 120 s. The plot of $\ln(M(\infty) - M(t))$ versus recovery time is nonlinear for times less than 10 s, indicating nonexponential recovery of magnetization. However, for times greater than 10 s the recovery of magnetization is exponential, and analysis yields a $\langle T_1 \rangle$ of 82 ± 10 s. Although nonexponential recovery of magnetization can be produced by a variety of motional mechanisms,³⁹ nonexponential recovery was also observed at the perpendicular edges of the powder pattern. In addition, acquisition of ²H-NMR echos at high repetition rates (i.e., one acquisition per second) yielded a highly averaged powder pattern, while data acquisition at much slower rates (one acquisition every 3 min) yielded the essentially rigid powder pattern shown in Figure 2a. An explanation for these observations might be heterogeneity of the sugar rings in the nucleoside sample. However, because the vast majority of the signal comes from the essentially immobile fraction, integrated spectral intensities indicated that 95% of the 2'' deuterons in the sample have $\langle T_1 \rangle$'s ≥ 70 s, the dynamics of the small mobile fraction were not considered further.

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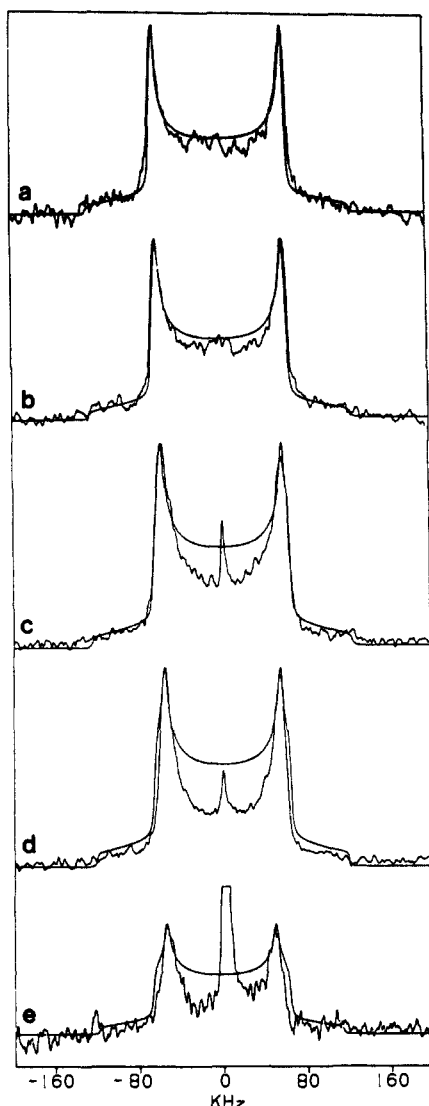


Figure 3. Solid-state ^2H -NMR line shape as a function of hydration level for dry DNA (a) and DNA equilibrated at 66% RH ($W = 4.1$) (b), 80% RH ($W = 10.4$) (c), 88% RH ($W = 15.3$) (d), and 92% RH ($W = 28.3$) (e). Solid lines are theoretical spectra calculated assuming a two-site jump model at rates in the fast regime with the jump angle varying from $\pm 9^\circ$ for dry DNA to $\pm 16^\circ$ for DNA equilibrated at 92% RH and static parameters of $\text{QCC}_{\text{static}} = 178$ kHz and $\eta_{\text{static}} = 0.0$.

Simulation of the experimental line shape is shown in Figure 2b and yields an effective asymmetry parameter η_{eff} of 0.02 and an effective deuteron quadrupole coupling constant QCC_{eff} of 172 kHz. Static quadrupolar coupling constants have been measured by NQR for a variety of organic compounds.^{41,42} In general, deuterons bonded to saturated, aliphatic carbons in rigid, linear molecules have $\text{QCC}_{\text{static}}$ values ranging from 167 ± 1.65 to 170 ± 1.3 kHz.⁴² In cyclic systems, however, $\text{QCC}_{\text{static}}$ values may be somewhat higher with 173.7 ± 1.7 kHz reported for cyclohexane⁴¹ and 178.5 ± 1.3 kHz for tetradeuterofuran.⁴² Static asymmetry parameters for deuterons bonded to saturated, aliphatic carbons are quite small, an η_{static} of less than 0.01 being reported for cyclohexane, for example. Assuming a $\text{QCC}_{\text{static}}$ of 173.7 kHz, the spectrum in Figure 2a can be simulated by assuming a two-site libration with an amplitude of $\pm 4^\circ$. Assuming the larger $\text{QCC}_{\text{static}}$ of 178 kHz requires that the libration amplitude be increased somewhat to $\pm 9^\circ$ (see Figure 2c). Both calculations assume a static asymmetry parameter of zero. If the assumption of a zero

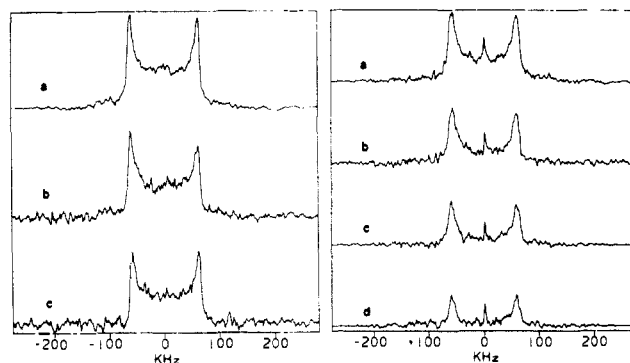


Figure 4. ^2H -NMR quadrupole echo spectra as a function of pulse spacing: (left-hand side) DNA equilibrated at 66% RH with pulse spacing varied with the values of (a) 50, (b) 70 and (c) 90 μs and (right-hand side) DNA equilibrated at 80% RH. The same progression of pulse spacing with (d) is shown corresponding to 120 μs .

static asymmetry parameter is not made, the resulting libration amplitudes will be smaller since by definition η must be ≥ 0 . More complex motional models may also be considered. If, for example, a biaxial model (a jump between four sites in two orthogonal directions) is assumed with a $\text{QCC}_{\text{static}}$ of 178 kHz, the resulting librational amplitudes θ and ϕ will be $\pm 12^\circ$ and $\pm 4^\circ$, respectively, with a root-mean-square amplitude of 8.9° . Assuming that the dynamics of the $2''$ deuteron is described by a two-site libration, the correlation time of the libration τ_c may be calculated from the observed $\langle T_1 \rangle$.³⁹ Assuming a $\text{QCC}_{\text{static}}$ of 173.7 kHz and a jump angle of 4° , the correlation time in the fast motion limit ($\tau^2\omega^2 \ll 1$) is 1.05 ps. In the slow motion limit ($\tau^2\omega^2 \gg 1$) the correlation time is 1.69×10^{-7} s. If the higher $\text{QCC}_{\text{static}}$ value of 178 kHz is assumed with a libration amplitude of 9° , the fast limit correlation time decreases to 0.204 ps, and the slow limit correlation time increases to 8.41×10^{-6} s.

Furanose Ring Dynamics in Hydrated [d-(CGCGA*A*TTTCGCG)]₂. The ^2H -NMR spectrum of the DNA dodecamer [d(CGCGA*A*TTTCGCG)]₂ deuterated at the $2''$ position on A5 and A6 is shown in Figure 3 as a function of hydration level. The corresponding ^2H NMR spectral and relaxation parameters are given in Table 1. The water content profile (the number of mol of water per mol of nucleotide W versus relative humidity RH) of this sugar-deuterated dodecamer is essentially the same as previously reported for base-deuterated DNA dodecamers.^{27,28} Spectral data in Table 1 indicate a decrease in QCC_{eff} from 172 kHz in dry samples to 146 kHz in samples equilibrated at 92% RH. The effective asymmetry parameter η_{eff} remains about constant at 0.04–0.06. As will be discussed below, the modest decrease in QCC_{eff} over the observed range of hydration suggests that any fast regime motions at the $2''$ sites on A5 and A6 must be low in amplitude. Large amplitude motions ($\gg 20^\circ$) in the fast rate regime would tend to produce far more drastic averaging of the EFG tensor, and consequently of the ^2H line shape, than would be suggested by the experimental data. However, the 30-fold decrease in $\langle T_1 \rangle$, the 3-fold decrease in $\langle T_{2c} \rangle$, the decrease in QCC_{eff} over the observed hydration range, and the distortion of the quadrupole echo spectral line shape as a function of pulse spacing at high levels of hydration suggest the occurrence of non-negligible dynamics at the $2''$ sites of A5 and A6.

Line shape trends shown in Figure 3 generally support the existence of significant motions of the $2''$ sites of A5 and A6 in the slow and/or intermediate rate regimes at high levels of hydration. As indicated in Table 1, QCC_{eff} decreases from 172 to 146 kHz as W increases from 0 to 28.3 (92% RH), a trend that may be reproduced by a number of N-site jump or diffusive models occurring at rates in the fast regime. However, ^2H line shapes obtained from samples equilibrated at $\geq 80\%$ RH consistently show less intensity in the center of the powder pattern than would be predicted assuming that the internal dynamics can be described by small amplitude, fast motions (Figure 3c–e), a trend also observed in base-deuterated dodecamers. Such distortions of

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quadrupolar echo spectra are known to be produced by anisotropic motions in the slow/intermediate rate regimes.^{43,44} Further evidence indicating the onset of internal motion at slow/intermediate rates is obtained by studying the quadrupolar echo line shape as a function of pulse spacing. Variation of the echo pulse spacing from 50 to 120 μ s produced little change in the ^2H powder pattern shapes obtained from samples equilibrated at 0 or 66% RH but produced severe distortions in the spectra of samples equilibrated at $\geq 80\%$ RH (Figure 4). Similar trends were also observed in the spectra of base-deuterated DNA dodecamers.²⁷

Although changes in the ^2H powder pattern shape as a function of hydration level for 2''-deuterated and base-deuterated DNAs are similar in a number of respects, there are also a number of differences. In base-deuterated DNA dodecamers QCC_{eff} decreases precipitously from 170 kHz at 88% RH to 71 kHz at 92% RH, whereas the QCC_{eff} of 2''-deuterated DNA dodecamers shows much less averaging over the same hydration range (Figure 3 and Table 1). Even more interesting is the comparative loss in spectral intensity as a function of hydration for base-deuterated and 2''-deuterated DNA dodecamers. In base-deuterated DNA the relative spectral intensities for the hydration series (RH) 66%:80%:88% was 1.0:~0.7:~0.2,²⁷ a 5-fold loss, whereas over the same hydration range, the relative spectral intensities obtained from 2''-deuterated samples were 1.0:~0.77:~0.74, indicating a reduction in relative spectral intensity of only 25%. In the following section, these observations will be evaluated with the goal of developing a motional model that describes the local dynamics of the sugar ring as well as the whole-molecule dynamics in a way consistent with studies of base and methyl-deuterated DNA dodecamers.

Discussion

Line Shape Analysis. At low hydration levels ($W = 0\text{--}4.1$), the dynamics of the 2'' deuterons on A5 and A6 may be described by simple jump or diffusive models with rates in the fast regime. Simulation of the ^2H powder pattern of the lyophilized 2''-deuterated DNA dodecamer yields a QCC_{eff} of 172 kHz with an η_{eff} of 0.04. Similarly, simulation of the ^2H powder pattern of the same sample equilibrated at 66% RH ($W = 4.1$) results in a QCC_{eff} of 170 kHz and an η_{eff} of 0.05. Assuming a $\text{QCC}_{\text{static}}$ of 178 kHz and an η_{static} of zero, as determined from studies of 2'-deoxyadenosine-2''-d₁, described above, and assuming the dynamics of 2'' sites at A5 and A6 can be described as a 2-fold jump, the ^2H powder line shapes (Figures 3a,b) may be reproduced by a librational amplitude of $\pm 9^\circ$ in dry DNA increasing slightly to $\pm 10^\circ$ at 66% RH. QCC_{eff} decreases from 161 kHz at 80% RH to 155 and 145 kHz at 88% and 92% RH, respectively, a trend that can be reproduced by assuming an increase in the librational amplitude from $\pm 13^\circ$ at 80% RH, to $\pm 14^\circ$ at 88% RH and $\pm 16^\circ$ at 92% RH but the line shapes obtained at these hydration levels are not reproduced (Figure 3c-c).

Other models may be considered to describe internal motions in DNA. The biaxial jump model described above can also reproduce the QCC_{eff} trend observed in Figure 3 and Table 1. Biaxial jump amplitudes θ and ϕ are found to be $\pm 12^\circ$ and $\pm 4^\circ$ in dry DNA (rms = 8.9°), $\pm 13^\circ$ and $\pm 4^\circ$ at 66% RH (rms = 9.6°), $\pm 18^\circ$ and $\pm 8^\circ$ at 80% RH (rms = 13.9°), $\pm 18^\circ$ and $\pm 14^\circ$ at 88% RH (rms = 16.1°), and $\pm 22^\circ$ and $\pm 18^\circ$ at 92% RH (rms = 20°). Like the two-site jump model, however, the biaxial jump model does not reproduce the line shapes shown in Figure 3c-e.

Brandes et al.^{45,46} have proposed the use of a diffusion-in-a-cone model to describe the restricted motion of purine-labeled polynucleotides. This model produces symmetrically averaged EFG tensors ($\eta_{\text{eff}} = 0$), if the motion occurs in the fast rate regime and if the static asymmetry parameter is zero. In the current study, as in the study of base and methyl-deuterated DNA dodecamers,²⁸

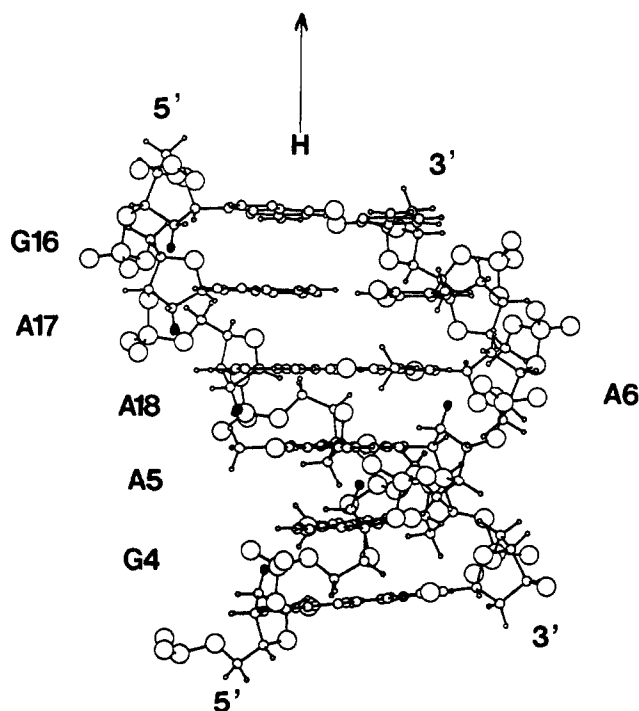


Figure 5. Orientation of the C2'-D2'' bond axis relative to the helix axis for the 2'-endo conformation. The 2'' deuterons of G4, A5, A6, G16, A17, and A18 are indicated by darkened circles. The helix axis is assumed to be normal to the base planes, and its direction is indicated by the vector H.

deviations of observed ^2H line shapes from axial symmetry imply that cone diffusion cannot account for all the fast internal dynamics in oligonucleotides. However, by ignoring deviations from axial symmetry and defining θ_0 as the half angle of the cone in which the C-D bond axis is allowed to freely diffuse, the amplitude of local motions may be estimated for this model assuming the effective quadrupolar coupling constant QCC_{eff} is given by

$$\text{QCC}_{\text{eff}} = (e^2qQ/h)_{\text{static}} S_{zz}$$

where the uniaxial order parameter S_{zz} for diffusion-in-a-cone is given by

$$S_{zz} = (\cos \theta_0 + \cos^2 \theta_0) / 2$$

Assuming that cone diffusion describes the sugar ring dynamics, the model yields $S_{zz} = 0.97$ and $\theta_0 = 11.5^\circ$ in the dry dodecamer, $S_{zz} = 0.96$ and $\theta_0 = 14.1^\circ$ at 66% RH, $S_{zz} = 0.90$ and $\theta_0 = 21.5^\circ$ at 80% RH, $S_{zz} = 0.87$ and $\theta_0 = 24.5^\circ$ at 88% RH, and $S_{zz} = 0.82$ and $\theta_0 = 28.9^\circ$ at 92% RH.

Dynamical models based on small amplitude motions in the fast rate regime cannot account for ^2H line shapes observed at higher hydration levels. In particular, the central part of the experimental powder patterns are consistently too low in intensity, but this feature can be simulated by assuming the presence of internal motions occurring at rates on the order of $10^4\text{--}10^5$ Hz. To develop a model of this intermediate regime motion, we first recall the observation stated above that the quadrupolar echo intensity of the sugar-deuterated sample diminishes by only 25% in going from $W = 4.1$ (66% RH) to $W = 10.4$ (88% RH), an observation that is in contrast to studies of base-deuterated DNAs where much greater attenuations of echo intensity have been observed.²⁷ We may resolve these seemingly disparate data with the following model. Assuming that the principal axis of the EFG tensor of the D6 aromatic deuteron is approximately perpendicular to the helix axis (see Figure 5) and the principal axis of EFG tensor of the 2'' deuteron makes a small ($\leq 30^\circ$) angle with the helix axis, the difference in echo intensity as a function of hydration for 2''-deuterated DNAs and base-deuterated DNAs may be explained if the intermediate regime motion is assumed to be a restricted reorientation about the helix axis. The effects of ^2H quadrupole

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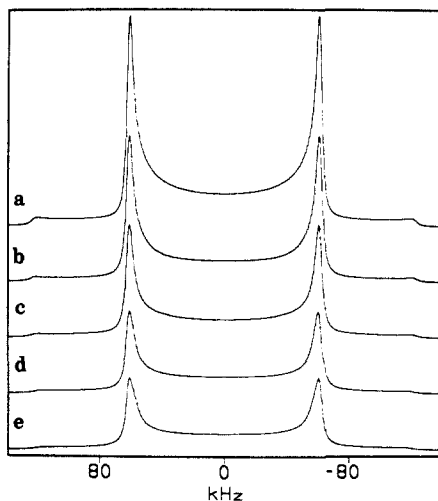


Figure 6. ²H-NMR line shapes ($QCC_{\text{static}} = 178$ kHz) for restricted reorientation about the helix axis modeled as a six-site jump ($N = 6$) with $\theta_{ij} = 20^\circ$ and a rate of 10^4 Hz and displayed as a function of the angle between the C-D bond and the helix axis: (a) 10° , (b) 20° , (c) 30° , (d) 50° , and (e) 90° .

echo line shape for various cone angles (i.e., the angle between the C-D bond and the helix axis: an angle of 0° when the ²H EFG tensor is parallel to the helix axis and an angle of 90° when the ²H EFG tensor is perpendicular to the helix axis) are shown in Figure 6, and it is evident that as the cone angle is increased the helix twist motion becomes more effective in modulating the EFG tensor. Fiber diffraction studies⁴⁷⁻⁴⁹ indicate that as DNA is progressively hydrated from $W = 4.1$ to $W = 10.4$, the transition from A to B form occurs, and we assume that at $W = 10.4$ the DNA is sufficiently hydrated to allow the onset of helix twisting motions at rates on the order of 10^4 Hz. This motion in turn produces an attenuation of the quadrupolar echo amplitude which is maximal for base deuterons and less extreme for 2'' deuterons if the sugar rings exist in conformations in which the C-D vector makes a moderate angle ($<30^\circ$) with the helix axis. In fact, the angle between the C2'-D2'' bond axis and the helix axis is sensitive to the conformation of the sugar ring. As shown in Figure 5 for the dodecamer $[d(\text{CGCGAATTCGCG})]_2$ in the ideal B form, where the sugar rings are in the C2'-endo conformation, the C2'-D2'' bond axis of A5 makes an angle of about 20° with the helix axis. In A form DNA, on the other hand, where the sugar rings are in the C3'-endo conformation, the same bond axis makes an angle of about 90° with the helix axis.

Restricted diffusion about the helix axis may be approximated as an N site jump process, where only nearest neighbor jumps are allowed.^{50,51} Then the relationship between the jump rate k , the arc angle θ_{ij} between jump sites i and j , and the average time τ required to reorient about the helix axis by one radian is given by

$$\tau = 1/k\theta_{ij}^2$$

which in turn yields the diffusion coefficient

$$D = 1/2\tau = k\theta_{ij}^2/2$$

The true diffusion limit is approached as N approaches infinity and θ_{ij} commensurately becomes very small. As in our studies of base-labeled DNA dodecamer,^{2,17,28} the number of sites N was

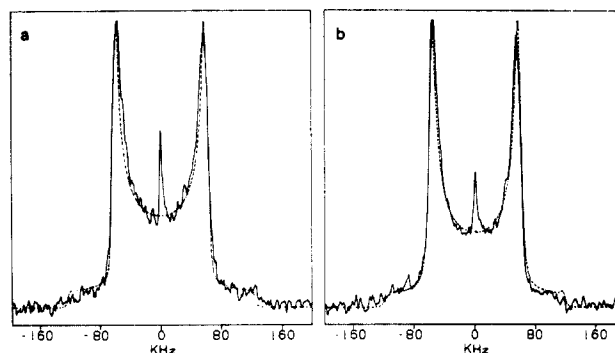


Figure 7. (a) ²H-NMR line shape for DNA equilibrated at 80% RH. Broken line corresponds to a dynamical model of restricted reorientation about the helix axis, $N = 6$, $\theta_{ij} = 5^\circ$, $k = 6.9$ kHz. (b) ²H-NMR line shape for DNA equilibrated at 88% RH. Broken line corresponds to a model of restricted reorientation about the helix axis, $N = 6$, $\theta_{ij} = 20^\circ$, $k = 13$ kHz.

typically limited to 6 in order to minimize computational time. Increasing N beyond 6 produced subtle line shape changes, but these changes were not considered significant given the limited signal-to-noise of our spectral data.

At low hydration levels, where torsional motions about the helix axis are negligible, the orientation of the librational axis of the sugar ring cannot be determined in a polycrystalline sample because the EFG tensor is uniaxial ($\eta_{\text{static}} = 0$). At higher levels of hydration, however, the mutual orientation of the helix axis and the axis of libration of the C2'-D2'' bond becomes important. To proceed further we assume that the axis of local libration of the C2'-D2'' bond is perpendicular to the helix axis. The reduction of QCC_{eff} to 161 kHz at 80% RH can then be simulated by assuming a two-site libration about this perpendicular axis with an amplitude of $\pm 13^\circ$. To invoke a slow rotation of the helix axis we assume the sugar ring exists in a conformation such as C2'-endo, where the C2'-D2'' bond makes an angle of about 20° with the helix axis. NMR studies of methyl-deuterated dodecameric DNA²⁸ indicate that at 80% RH torsional motions about the helix axis may be modeled as a six-site jump ($N = 6$) with a θ_{ij} of about 5° at a rate of 1 kHz. Studies of that same dodecamer deuterated at the H6 site on T7 similarly obtain a θ_{ij} of 5° at a slightly higher rate of 3.3 kHz. As shown in Figure 7a essentially the same model of torsional motions applies to 2''-deuterated DNA dodecamers with the line shape of the 80% RH data being successfully reproduced assuming $\theta_{ij} = 5^\circ$ at a rate of 6.9-9.8 kHz. Assuming a slightly larger value of jump angle $\theta_{ij} = 10^\circ$ results in a best fit for rates 4.9-6.9 kHz.

Samples hydrated at 88% RH absorbed ~ 15 molecules of water per nucleotide, and the DNA assumed a gel-like consistency. As indicated in Table I, QCC_{eff} decreases to 155 kHz at 88% RH from 161 kHz at 80% RH, a trend that can be reproduced by increasing the amplitude of fast libration to 14° . ²H NMR studies of methyl-deuterated DNA dodecamers equilibrated at 88% RH indicate that torsional dynamics about the helix axis can be simulated by using the same N-site jump models as in the 80% RH case, and by using somewhat larger values of θ_{ij} and slightly higher jump rates. This is a physically reasonable approach in view of the fact that further hydration of DNA above 80% RH simply adds water to spaces between DNA molecules allowing less restricted reorientation about the helix axis. As shown in Figure 7b, the line shape of DNA equilibrated at 88% RH can be simulated by assuming $\theta_{ij} = 20^\circ$ and a rate of about 9.8-13 kHz.

Hydration at 92% relative humidity results in DNA samples absorbing ~ 28 molecules of water per nucleotide. DNA at this hydration level is a viscous liquid in appearance and scatters light strongly. Studies of high molecular weight DNAs indicate that a magnetically aligned liquid crystal phase occurs at this hydration level,^{52,54} and a recent study of methyl-deuterated [d-

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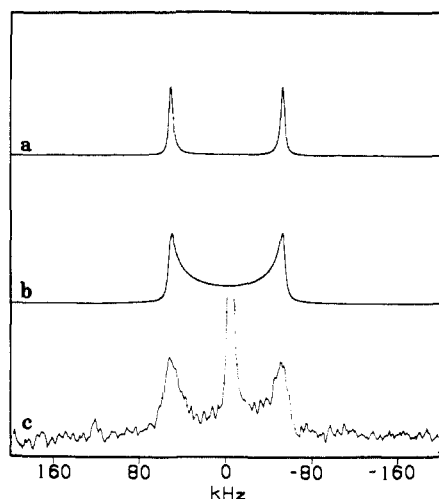


Figure 8. (a) Theoretical spectrum assuming formation of a liquid crystalline phase with 70% of the sample existing in ordered domains with the helix axes perpendicular to the magnetic field and a Gaussian ordering profile with $\sigma = 25^\circ$. A dynamical model of free diffusion about the helix axis at 100 kHz is assumed with a fast libration of the furanose ring about an axis perpendicular to the helix axis with an amplitude of 16° . (b) Similar model to (a) only including a helix wobble of 11° at a rate of 1.56 MHz and a reduced local libration amplitude of 14° . (c) ^2H -NMR spectrum of DNA equilibrated at 92% RH.

(CGCGAATTCGCG) $_2$ indicates that this material forms a liquid crystal phase as well.⁵⁵ At this hydration level, water molecules continue to fill the space between DNA molecules, and internal molecular motions would be expected to become even less restricted.

Simulation of the experimental ^2H NMR spectrum of $2''$ -deuterated DNA hydrated at 92% RH presents a considerable problem. ^2H NMR studies of methyl-deuterated [d-(CGCGAATTCGCG) $_2$] indicate that at a hydration level of $W = 26.6$, the DNA is partially ordered into liquid crystalline domains in which the helix axis is oriented perpendicular to the magnetic field, with about 70% of the DNA in liquid crystalline domains and the remaining 30% randomly ordered.²⁸ At a hydration level of $W = 29.6$ the fraction of randomly ordered DNA approaches zero. Internal motions in both domains were described by free diffusion about the helix axis at a rate of about 400 kHz, but the low signal-to-noise of the spectra of $2''$ -deuterated DNA at 92% RH precludes such detailed modeling of the degree of ordering and the internal dynamics. As shown in Figure 8a, however, assuming approximately the same degree of long-range ordering as in the methyl-deuterated sample together with internal motions described as a local libration of the furanose ring of $\pm 16^\circ$ and diffusion around the helix axis at a rate on the order of 100 kHz results in a rather poor fit to the experimental spectrum shown in Figure 8c.

It is not unreasonable, however, to assume that a DNA dodecamer may be undergoing restricted reorientations about axes other than the helix axis. In studies of methyl-deuterated DNA dodecamers,²⁸ a rough estimate of contributions from diffusion about an axis perpendicular to the helix axis was obtained from consideration of the dynamics of sphero-cylinders.^{56,57} Assuming the diameter of an oligonucleotide is about 24 Å and the length is given by $N \times 3.4$ Å, where N is the number of base pairs ($N = 12$ for a dodecamer), the length-to-diameter ratio of the DNA dodecamer is about 1.7. The theory of Tirado et al.^{56,57} indicates that the ratio of the diffusion coefficient for reorientation about an axis parallel to the helix axis D_{\parallel} to the diffusion coefficient for reorientation about an axis perpendicular to the helix axis D_{\perp} should equal about 1.9 for an oligonucleotide with a length-to-diameter ratio of 1.7. Including a slow helix wobble (restricted

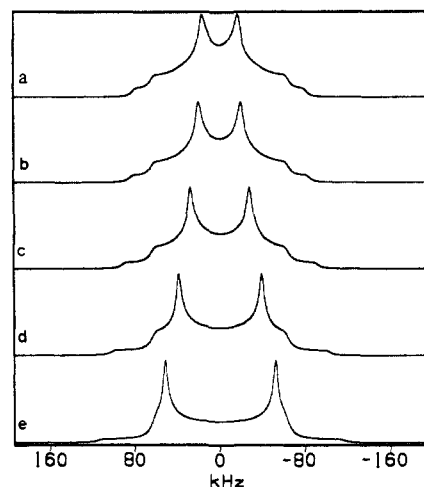


Figure 9. Theoretical line shapes for the $\text{C}2''\text{-D}2''$ bond undergoing a two-site jump between the $\text{C}2'$ -endo and $\text{C}3'$ -endo conformations at a rate of 10^9 Hz as a function of conformational populations: (a) 0.5/0.5, (b) 0.6/0.4, (c) 0.7/0.3, (d) 0.8/0.2, (e) 0.9/0.1. In addition, the oligonucleotide is assumed to undergo a restricted reorientation about the helix axis, modelled as a six-site jump ($N = 6$), with $\theta_{ij} = 20^\circ$, at a rate of 10^4 Hz.

rotation about an axis perpendicular to the helix axis) with an amplitude of 22° and a rate of 1.56 MHz in addition to unrestricted diffusion about the helix axis at a rate of 400 kHz produces slightly improved fits of experimental spectra of methyl-deuterated DNA dodecamers at hydration levels above 90% RH. Including essentially the same wobbling motion in a fit of $2''$ -deuterated DNA results in substantially improved fits to experimental data, except that QCC_{eff} becomes too small (< 146 kHz) if the amplitude of local libration is assumed to be 16° . Reducing the libration amplitude to the value at 88% RH ($\pm 14^\circ$) resulted in the spectrum shown in Figure 8b.

Throughout this discussion, the approach taken in analyzing ^2H -NMR spectra of $2''$ -deuterated DNA dodecamers has been to disallow the occurrence of large amplitude, fast motions on the basis of large observed values of QCC_{eff} . The validity of this assumption should be considered in detail given the large body of literature, cited above, in which is reported the occurrence of interconversions of the furanose rings in nucleosides, oligonucleotides, and high molecular weight DNAs between $\text{C}2'$ -endo and $\text{C}3'$ -endo conformations, at time scales on the order of nanoseconds. If the furanose rings of A5 and A6 were to interconvert between $\text{C}2'$ -endo and $\text{C}3'$ -endo, for instance, the jump would occur with an amplitude of over 60° . If such an interconversion were to occur on the nanosecond time scale between conformations with equal a priori probabilities, the drastically averaged line shape shown in Figure 9a would result, a line shape that has never been experimentally observed for the $2''$ -deuterated dodecamer at any hydration level. If, on the other hand, interconversions occur between conformers with unequal a priori probabilities, as has also been suggested in the literature, drastically averaged line shapes would still result, as shown in Figures 9b-e, where $2'$ -endo/ $3'$ -endo populations of 0.6/0.4, 0.7/0.3, 0.8/0.2, and 0.9/0.1 were assumed. Note that jumps between conformers in which the $\text{C}2''\text{-D}2''$ bond orientation changes by large angles on the time scale of nanoseconds invariably produce line shapes with larger η_{eff} values (≥ 0.1) than have been observed experimentally. However, as indicated by Figure 9e, the presence of small populations of $3'$ -endo (≤ 0.1) may not be detectable from the ^2H line shape.

The theory of Altona and co-workers predicts an interconversion between $\text{C}2'$ -endo and $\text{C}3'$ -endo on the time scale of nanoseconds. It seems reasonable to assume that such high rates of conformational interconversion would apply to solid samples of DNA equilibrated at high relative humidities, where hundreds of waters of hydration are associated with a single DNA dodecamer (at 92% RH, for example, DNA samples absorb about 700 waters per dodecamer). However, for completeness, the possibility of lower

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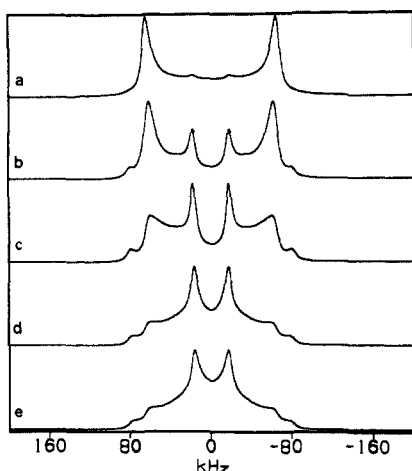


Figure 10. Theoretical line shapes for the C2'-D2'' bond undergoing a two-site jump between the C2'-endo and C3'-endo as a function of jump rate (Hz): (a) 10^4 , (b) 10^5 , (c) 10^6 , (d) 10^7 , and (e) 10^8 . In addition, the oligonucleotide is assumed to undergo a restricted reorientation about the helix axis, modelled as a six-site jump ($N = 6$), with $\theta_j = 20^\circ$, at a rate of 10^4 Hz.

rates of interconversion must be considered. Figure 10 shows the averaged ^2H line shapes produced by interconversion rates of 10^4 , 10^5 , 10^6 , 10^7 , and 10^8 Hz, assuming equal a priori probabilities. Even if unequal probabilities are assumed, drastically averaged line shapes are produced that have not been experimentally observed at any hydration level. The ^2H line shape becomes insensitive to conformational interconversions when rates decrease to 10^4 Hz, however, so ^2H NMR line shape data alone cannot exclude the possibility of such interconversions occurring at rates $\leq 10^4$ Hz. However, a recent study of hydrated calf thymus DNA by Harbison and co-workers⁵⁸ using ^{13}C magic angle spinning indicates absence of large amplitude motions of the furanose rings at longer time scales as well.

Spin-Lattice Relaxation Data. Brandes et al.⁴⁶ have measured both the Zeeman relaxation time T_{1Z} and the quadrupolar relaxation time T_{1Q} at two different values of static magnetic field strength, which enables a study of the individual spectral densities $J_1(\omega_0)$ and $J_2(2\omega_0)$ as a function of frequency. In this study, T_{1Z} at a single field strength has been measured, and although we cannot present as complete an analysis of the dynamics of the $2''$ -deuterated DNA dodecamer as has been applied to purine base motion in high molecular weight DNA, a comparison of trends in Zeeman relaxation as a function of hydration level with earlier studies of base-deuterated DNAs is possible.

Zeeman spin-lattice relaxation time (T_1) for the $2''$ -deuterated DNA dodecamer and the H8-deuterated dodecamer are plotted as a function of hydration level in Figure 11. (T_1) for the $2''$ deuterons of A5 and A6 is 1.8 s in the dry, lyophilized dodecamer, which is about equal to the value of 1.66 s reported by Kintanar et al.²⁷ for the H8-deuterated dodecamer and somewhat shorter than the 7 s reported by Brandes et al. for H8-deuterated high molecular weight Li-DNA.⁴⁵ Hydration at 66% RH resulted in a moderate decrease in the (T_1) of the $2''$ -deuterated dodecamer to 1.04 s in comparison to the hydrated H8-deuterated dodecamer which decreased to 0.78 s and the H8-deuterated high molecular weight Li-DNA which decreased to 0.30 s. In going from 66% to 80% RH, (T_1) of both the H8-deuterated and the $2''$ -deuterated DNA dodecamers decreased markedly to values of 0.11 and 0.24 s, respectively. Mai et al.⁵⁹ in a ^{31}P NMR study of the backbone dynamics of the Na salt of calf thymus DNA observed a similar decrease in the ^{31}P spin-lattice relaxation time and suggested that the transition from A to B form DNA occurs with an increase in internal motion, thus accounting for the reduction of T_1 . Brandes et al.⁴⁵ in a ^2H -NMR study of both the Na and the Li

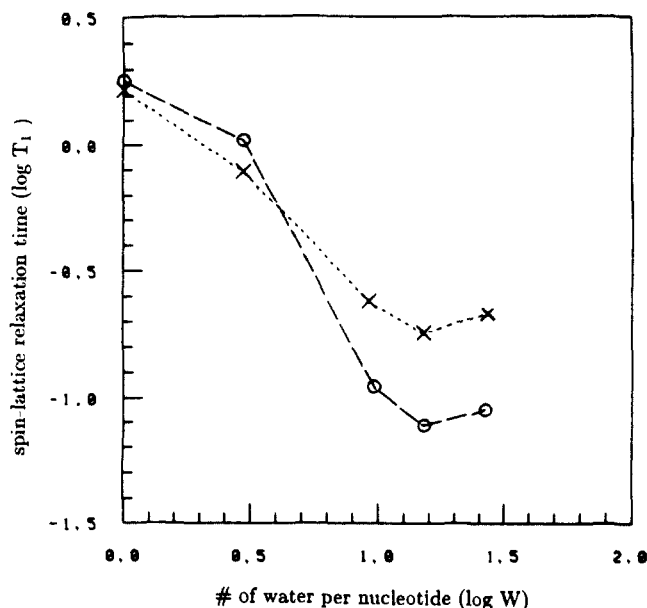


Figure 11. ^2H spin-lattice relaxation time (T_1) for H8-deuterated (\times) and $2''$ -deuterated (\circ) DNA as a function of hydration level (W = number of water molecules per nucleotide).

salts of high molecular weight DNA also observed a similar decrease in T_1 but disagreed with the attribution of this T_1 decrease to the onset of the new motional regime caused by conversion to B form DNA which produced this T_1 decrease since their Li-DNA remained in the B form throughout the hydration range covered by their study.

Brandes et al.^{45,46} also noted that, up to a hydration level of $W = 20$, the ratio of the ^{31}P and ^2H spin-lattice relaxation times is approximately constant, implying that base and backbone motions are coupled. In fact, inspection of Figure 11 shows that the (T_1) values for H8-deuterated and $2''$ -deuterated DNA dodecamers also vary in constant proportion as a function of hydration level between 80% and 92% RH, implying a similar coupling between base and furanose ring motions in DNA dodecamers. Models incorporating collective torsional modes into the internal dynamics of DNA have long been used to explain FPA and EPR data of DNA solutions^{4,60} and may be invoked to explain the apparent coupling of the base and furanose ring motions in DNA dodecamers as well. In this picture, the increased amplitudes of the fast regime motions of the C-D bond vectors on the base and furanose rings at high levels of hydration may be due in part (or entirely) to collective motions of the polymer. In a recent study, Brandes et al. note that the large ratio of $J_1(\omega_0)$ to $J_2(2\omega_0)$ measured for H8-deuterated high molecular weight DNA excludes the possibility that in-plane torsional motion is the dominant relaxation mechanism in DNA for hydration levels up to 84% RH ($W = 13$) but note that the ratio decreases at higher hydration levels. The possibility that coupled torsions are responsible for the (T_1) trend observed in Figure 11 remains. We note that studies of the frequency dependence and the dependence of ^2H relaxation on oligonucleotide length would be helpful in elucidating the nature of internal motions in synthetic DNAs. Such studies as well as relaxation and line shape studies of DNA oligomers selectively deuterated on the H5'/5''⁶² sites are in progress in this laboratory.

Conclusion

As with all model-based studies, this work cannot identify a unique model to explain the ^2H line shapes observed for selectively deuterated oligonucleotides. However, it is possible to confirm

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the validity of certain models and to eliminate models which produce theoretical line shapes that do not agree with experiment. Hence, although we do not claim to have identified a unique model of fast regime motions, and in fact have calculated QCC_{eff} for a variety of models of fast motion of the furanose rings, our data do constrain the amplitudes of these motions to be small. Models which propose large amplitude conformational interconversions of the furanose rings in oligonucleotides on the nanosecond time scale do not appear to be valid in the light of our data, at least over the hydration range studied, although the presence of small populations of minor conformers cannot be discounted on the basis of ^2H NMR line shape analysis.

Neither do we claim that restricted reorientation about the helix axis is the only model which explains the ^2H line shape trends above 80% RH. In fact, certain aspects of our spectral data (i.e., the ^2H line shape observed at 92% RH) can only be explained by invoking reorientations about additional axes in accordance with well-established theories of the dynamics of rigid cylinders.^{56,57} In general, our model of restricted reorientational motions about the helix axis and additional axes has accurately reproduced the trends in ^2H experimental line shape observed for DNA dodecamers deuterated on the bases and the furanose rings, and so we claim it to be a reasonable, though not unique model for internal dynamics in DNA dodecamers. A summary of our ^2H NMR study of the furanose ring dynamics of the DNA dodecamer [d(CGCGAATTCGCG)]₂ follows: (1) The amplitude of fast regime motions of the furanose rings of A5 and A6, as elucidated by the effective quadrupolar coupling constant QCC_{eff} increases from about 9° in dry, lyophilized samples to about 16° in samples equilibrated at 92% RH ($W = 28.3$), assuming a simple two-site jump model, a QCC_{static} of 178 kHz, and an asymmetry parameter of zero. Other simple jump or restricted diffusion models similarly yield small motional amplitudes. Assumption of a smaller QCC_{static} would moderate these amplitudes even further as would the assumption of a static symmetry parameter greater than zero. For example, if a QCC_{static} of 173.7 kHz is assumed, the amplitude of the fast regime motion of the furanose ring (modeled as a two-site jump) is only 3–4° in the dry dodecamer, $\approx 5^\circ$ for the dodecamer at 66% RH, $\approx 9^\circ$ at 80% RH, $\approx 11^\circ$ at 88% RH and $\approx 13^\circ$ at 92% RH. (2) Studies of ^2H Zeeman relaxation as a function of hydration level indicate that above 80% RH, the motions of the bases and furanose rings appear to be coupled,

implying that part, or all, of the decrease in QCC_{eff} may be due to overall motion of the oligomer, as opposed to an increase in the amplitude of localized motions of the furanose rings. (3) Over the hydration range studied, there is no ^2H NMR evidence to suggest the occurrence of an interconversion between the C2'-endo and C3'-endo conformations (or any other conformations where the change in amplitude is greater than 16°) of the furanose rings of A5 and A6 on the nanosecond time scale or at any greater than 10^4 or 10^5 Hz. (4) Changes in the ^2H line shape over the hydration range 66% RH ($W = 4.1$) to 92% RH ($W = 28.1$) can be accounted for by a modest increase in the amplitude of fast regime motions of the furanose rings together with the onset of reorientations about the helix axis, which increase in amplitude and rate with hydration level. Models of restricted motion about the helix axis used to fit the line shapes of 2'-deuterated DNA oligomers at 80% and 88% RH are in excellent agreement with models used in similar studies of methyl-deuterated DNA dodecamers. Rates of restricted reorientation about the helix axis derived from line shape studies of 2'-deuterated and methyl-deuterated DNA dodecamers are also in agreement with similar studies of H8-deuterated DNA dodecamers hydrated at 80% RH but differ by an order of magnitude for H8-deuterated dodecamers hydrated at 88% RH. Line shape simulations in this latter case, however, were of spectra of very low signal-to-noise, and derived rates were only approximate. (5) Line shape data of 2'-deuterated DNA dodecamers hydrated at 92% RH are of limited signal-to-noise and therefore preclude detailed modeling of the motion of the furanose rings. However, the large QCC_{eff} eliminates the possibility of large amplitude motions on the nanosecond time scale, and the general line shape is consistent with the formation of a liquid crystalline phase.

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