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Dynamics of Bases in Hydrated $[d(CGCGAATTCGCG)]_2$†

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ABSTRACT: Solid-state ^2H NMR spectroscopy has been used to investigate the dynamics of a DNA oligonucleotide with a defined sequence, $[d(CGCGAATTCGCG)]_2$, which contains the *Eco*RI binding site. Quadrupole echo line shapes and spin–lattice relaxation times were obtained as a function of hydration on two different deuteriated samples, both in the form of the Na salt. In one sample, the C8 protons of all purines in the self-complementary dodecamer were exchanged for deuterons. In the other sample, a specifically labeled thymidine (C6 deuteriated) was synthetically incorporated at the seventh position (counting 5′ to 3′) in the sequence. The general trends for both samples were quite similar. At all levels of hydration, the data reveal the presence of a rapid, small-amplitude libration of the bases ($\tau_c \leq 1$ ns, 6°–10° amplitude). At the higher hydration levels (80% relative humidity or higher), the results indicate the presence of a much slower motion ($\tau_c \sim 10$–100 μs), which at 80% relative humidity is of small amplitude (~5°) and at higher hydration levels may be of larger amplitude. There is no evidence for large-amplitude (greater than ±10°) motion on a nanosecond or faster time scale under any hydration condition. The ^2H NMR results were analyzed with a dynamical model which treats the oligonucleotide as a deformable filament and which can include collective torsional fluctuations. The slow motion observed at high hydration levels is attributed to the uniform twisting mode (of the entire helix). Evidently, the various torsional modes decay more slowly in the viscous hydrated solid sample than in solution. The higher order (collective) torsional modes are predicted to occur on a nanosecond time scale and can account for the significantly reduced T_1 value found at high hydration levels.

The view of DNA as a rigid, uniform double helix has changed dramatically in the last few years. The determination of X-ray crystal structures of several synthetic DNA oligomers (Wing et al., 1980; Drew et al., 1981; Wang et al., 1982; Shakked et al., 1981) reveals that while these molecules have overall geometries similar to those of the A and B forms of DNA previously characterized by fiber diffraction techniques (Arnott & Hukins, 1972), there is considerable sequence-dependent variability of the local structure (Dickerson & Drew, 1981; Dickerson, 1983). Studies utilizing two-dimensional NMR methods indicate that such sequence-dependent structural microvariability also exists in DNA oligonucleotides in solution (Nerdal et al., 1988). An intriguing prospect is that this phenomenon provides the molecular basis for the specificity of protein–DNA interactions. Therefore, it is important to determine how well-defined are these structures and what is the range of allowed deviations from the average structure. In other words, what are the dynamics of DNA molecules?

Fortunately, scientific inquiry has not lagged in this area, and a large variety of physicochemical techniques has been used to study the motion of DNA in solution and in the solid state. The single-crystal X-ray diffraction data have been analyzed with atomic isotropic thermal factors (Drew et al., 1981, 1982) and also an anisotropic segmental rigid-body model (Holbrook & Kim, 1984) to extract information about the amplitudes and in some cases the direction of motion of the various moieties in the B-form DNA dodecamer. However, a full understanding of DNA dynamics requires the knowledge of the *rate* of motion, which is not available from the X-ray diffraction studies.

Several optical techniques have been used to study high molecular weight DNA including electric dichroism (Ding et al., 1972) and depolarized dynamic light scattering (Schmitz & Schurr, 1973; Schurr, 1977). These studies reveal that such high molecular weight DNA molecules are quite flexible, possessing slow coil-deformation (Rouse–Zimm) modes as well as more rapid end-over-end persistence length rotations. The

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latter motion has a characteristic decay time on the order of several microseconds in solution. These relatively slow, large-scale motions have essentially no effect on the local structure of DNA, however, and thus are not of significant concern here.

Faster motions in DNA have been detected with time-resolved fluorescence depolarization of ethidium bromide bound to DNA (Wahl et al., 1970; Thomas et al., 1980; Millar et al., 1980). These motions have been identified as collective torsional deformations of the helix (Barkley & Zimm, 1979; Allison & Schurr, 1979). Recent fluorescence anisotropy measurements with greater temporal resolution reveal a very fast small-amplitude decay with relaxation time $\tau \sim 100$ ps (Magde et al., 1983). This has been attributed to rapid, local wobbling of the dye in the intercalation site (Magde et al., 1983; Shibata et al., 1985), which presumably reflects the internal motion of base pairs in DNA.

Nuclear magnetic resonance spectroscopy has also been utilized extensively to characterize the dynamics of DNA in solution. The NMR relaxation data have been interpreted for the most part within the framework of a model that includes isotropic tumbling of the rod (end-over-end persistence length rotation) and local internal motions of various types (Hogan & Jardetzky, 1979, 1980; Bolton & James, 1979; Bendel et al., 1982; Bendel & James, 1983; Levy et al., 1983; Behling & Kearns, 1986; Kearns, 1987). These studies indicate the presence of large-amplitude ($\geq \pm 20°$) internal angular motions on a nanosecond time scale. However, the results of these NMR relaxation studies have recently been called into question because the analyses failed to include the effects of collective torsional motions. When these same NMR relaxation data are reevaluated with a model that includes these effects, the amplitudes of local internal angular motion that are required to fit the data are $\sim 10°$ or less (Allison et al., 1982; Langowski et al., 1985; Schurr & Fujimoto, 1988).

The major disadvantage of solution NMR investigations of dynamics is that the data give little indication of the *nature* of the motion. Any such information is lost because of the isotropoic averaging of the spin-interaction tensors by molecular tumbling (end-over-end persistence length rotation) of the molecules in solution. This is not the case in solid-state NMR, however, where the anisotropy of the spin interaction is preserved and expressed in the line shape. The line shape is extremely sensitive to the rate and type of motion affecting the relevant nuclear spin [for reviews, see Torchia (1984) and Opella (1986)]. In addition, the spin–lattice relaxation times (T_1) in the solid state are also sensitive to the rate and in some cases the type of motion (Torchia & Szabo, 1982). Moreover, the analysis of these data is actually simplified because molecular tumbling is insignificant and can be neglected.

Several solid-state NMR studies of DNA have been undertaken. These investigations have utilized either ^{31}P NMR to investigate the motion of the phosphodiester backbone linker or ^2H NMR to study the dynamics of the purine bases. The ^{31}P studies indicate that at low levels of hydration there is very little motion of the backbone but with increasing hydration there is a significant reduction in the breadth of the line and in the spin–lattice relaxation time (T_1), suggesting a large-amplitude motion of the phosphate group with a rate greater than 10^4 s^{-1} (Diverdi & Opella, 1981; Mai et al., 1983; Fujiwara & Shindo, 1985). The ^2H NMR studies on the other hand reveal only small-amplitude motions of the purine bases on a nanosecond time scale, even at very high hydration levels (Diverdi & Opella, 1981; Bendel et al., 1983; Brandes et al., 1986; Shindo et al., 1987). However, several of these studies also reveal a substantial decrease in the quadrupole echo ^2H NMR signal of highly hydrated DNA, which suggests significant motion on a much slower time scale, $\tau_c \sim 10$ μs.

Here, we report the first solid-state ^2H NMR study of a DNA oligonucleotide with a defined sequence, [d-(CGCGAATTCGCG)]$_2$. This self-complementary dodecamer contains the *Eco*RI binding site and is the same sequence investigated by Wing et al. (1980) in the first report of the single-crystal X-ray diffraction structure of B-form DNA. We present results on two different deuteriated samples of the same sequence in the form of the Na salt. In the first sample, the C8 protons of all purines in the dodecamer were exchanged for deuterons. In the second sample, a specifically labeled thymidine (C6 deuteriated) was synthetically incorporated at the seventh position (counting 5′ to 3′) in the sequence.

Deuterium NMR quadrupole echo line shapes and T_1 values of both samples were acquired as a function of hydration, and the data were analyzed to yield a detailed model of DNA motion. Similar to previous solid-state ^2H NMR studies of DNA, small-amplitude librations of the base were observed to occur on a nanosecond time scale. At high hydration levels, where the DNA is expected to be in the B-form, the data dictate the presence of an additional slower motion. We discuss in detail the nature of these motions and whether they are consistent with a model that includes collective torsional motions of the double helix. In addition, we compare the dynamics of DNA observed in the hydrated solid with that which may occur in solution. Finally, we compare the motion of the uniformly labeled deuteriopurine sample and the selectively labeled deuteriothymidine DNA and discuss the implications for obtaining sequence-dependent dynamical information.

Materials and Methods

Preparation of Labeled DNA. The DNA samples used in this study were synthesized by the solid-phase phosphite triester method using methyl phosphoramidites on an Applied Biosystems 380A DNA synthesizer. Each sample was synthesized twice on a 10-μmol scale, yielding over 1800 A_{260} units of crude material. Each synthesizer run was purified separately by ethanol precipitation followed by gel-exclusion chromatography on a superfine Sephadex G-25 column (Kintanar et al., 1987).

The uniformly 8-deuteriated DNA oligonucleotide [henceforth designated [U-$^2H_{12}$](CGCGAATTCGCG)$_2$] was prepared by deuterium exchange in slightly basic aqueous (D_2O) solution (Tomasz et al., 1972; Benevides & Thomas, 1985). The lyophilized, purified DNA sample from a single synthesizer run was dissolved in D_2O to preexchange the labile protons. A D_2O-based buffer was prepared as follows. Phosphate-buffered saline (20 mM Na_2PO_4, 100 mM NaCl, 1 μM EDTA,[1] pH adjusted to 8.0) was lyophilized, dissolved in 1 volume of D_2O, equilibrated for 30 min at 25 °C, relyophilized, and rediluted with D_2O (1 volume). The preexchanged DNA sample was dissolved in 5 mL of the D_2O-based buffer. The solution was heated to 75 °C in a preequilibrated heat block for 12 h, lyophilized, dissolved in H_2O to back-exchange the labile deuterons, and relyophilized to yield the desired uniformly 8-deuteriated oligonucleotide.

The selectively labeled DNA containing 6-deuteriated thymidine at T7 of the sequence [henceforth called [S-$^2H_2$](CGCGAAT*TCGCG)$_2$] was prepared with a labeled methyl phosphoramidite at the appropriate step during the

[1] Abbreviations: EDTA, ethylenediaminetetraacetic acid; DMT, dimethoxytrityl.

DNA synthesis. The deuteriated phosphoramidite was prepared by the following procedure. [6-$^2H_1$]Thymidine was first prepared by the method of Rabi and Fox (1973) as described in Kintanar et al. (1988). The labeled nucleoside was protected at the 5′-position with the acid-labile 4,4′-dimethoxytrityl group according to established procedures (Smith et al., 1962; Rammler & Khorana, 1962; Ti et al., 1982). The 5′-DMT-protected [6-$^2H_1$]thymidine was then converted to the corresponding [6-^{2}H]-3′-*O*-[methoxy(diisopropylamino)phosphinyl]-5′-(dimethoxytrityl)thymidine by the procedure of Barone et al. (1984). To an oven-dried 10-mL round-bottom flask fitted with magnetic stir bar was added 150 mg (0.31 mmol) of [6-$^2H_1$]-5′-(dimethoxytrityl)thymidine and 26 mg (0.15 mmol) of methyl phosphoroditetrazolide. The flask was sealed under argon, and the contents were diluted with 2 mL of CH_2Cl_2 (distilled from CaH_2). The resultant solution was treated with 87 mg (0.34 mmol) of bis(diisopropylamino)-methoxyphosphine (Barone et al., 1984) stirred at 25 °C for 1 h, and then poured over ice-cold saturated aqueous $NaHCO_3$. The two layers were separated, and the aqueous layer was extracted with two 10-mL portions of CH_2Cl_2 (alumina treated). The combined organic layers were washed with two 10-mL portions of ice-cold saturated $NaHCO_3$, dried over Na_2SO_4, and concentrated under vacuum to yield a white foam. The crude product was purified by flash chromatography (5% pyridine, 95% alumina-treated CH_2Cl_2) to yield 140 mg (0.22 mmol, 71%) of the desired labeled methyl phosphoramidite. The phosphoramidite was determined to be >95% pure by ^{31}P NMR (200 MHz) and ^{1}H NMR (500 MHz).

After the preparation and purification of the DNA samples, the purity and extent of deuteriation were verified by 500-MHz solution ^{1}H NMR spectroscopy. Both samples were at least 95% pure (free of short failure sequences and small-molecule contaminants) as indicated by comparison of the NMR spectra to the previously published NMR spectrum of the unlabeled sequence (Hare et al., 1983). NMR spectroscopy also indicated that the [U-$^2H_{12}$](CGCGAATTCGCG)$_2$ sample had >95% deuterium incorporation at all C8 positions and that the [S-$^2H_2$](CGCGAAT*TCGCG)$_2$ sample had ~90% deuterium incorporation at the C6 position in residue T7 of the sequence.

Prior to solid-state ^{2}H NMR spectroscopy, the DNA samples were desalted on a Sephadex G-10 column (60 cm × 2 cm) eluted with H_2O and then lyophilized. To the purified, desalted DNA was added 10% by weight sodium chloride. The samples were then dissolved in ^{2}H-depleted water and lyophilized. The dry DNA was packed into a 5 mm × 15 mm glass tube and hydrated by vapor equilibration against saturated salt solutions (in ^{2}H-depleted water) of known relative humidity (Weast, 1979). The samples were allowed to equilibrate for 2–3 weeks at a given relative humidity to ensure complete hydration, at which time they appeared macroscopically homogeneous. The actual water content of the DNA samples was not determined, but as the hydration protocol was quite similar to that described by Falk et al. (1962, 1963a,b) and Brandes et al. (1986), it was assumed that the samples contained about the same amount of water per nucleotide as reported by these workers.

Solid-State NMR Spectroscopy. Solid-state ^{2}H NMR spectra were obtained at 76.76 MHz on a home-built NMR spectrometer (Gladden and Drobny, unpublished results) using a quadrupole echo pulse sequence with an eight-step phase cycle (Griffin, 1981). Typically, the delay between pulses was 50 μs, and the dwell time was 900 ns. The 90° pulse length was 3 μs or less. Data acquisition was initiated prior to the top of the solid echo, and the time domain data were left shifted to the echo maximum prior to Fourier transformation. The ^{2}H NMR spectra were apodized with 2000-Hz Lorentzian line broadening.

Deuteron spin–lattice relaxation times (T_1) were determined with a saturation–recovery pulse sequence. The magnetization recovery was determined from the maximum amplitude of the quadrupole echo. The data were analyzed by both a linear least-squares routine and a nonlinear least-squares routine (De Fontaine et al., 1975).

Simulations of the ^{2}H NMR line shapes were obtained with a program described by Greenfield and co-workers which can accommodate various motional models (Greenfield et al., 1987). Static values of the quadrupole coupling constant (e^2qQ/h) and the asymmetry parameter (η) were obtained from the appropriate monomer studies (Tsang et al., 1987; Kintanar et al., 1988). The program was run on a DEC μVax II.

Spin–lattice relaxation times were calculated from different motional models. One model assumed only an internal 2-fold jump, and the expression of Torchia and Szabo (1982) was utilized. A second model included the effects of collective torsional motions (see Appendix). The total correlation function was taken to be the product of a twisting and an internal motion correlation function. The internal correlation function was taken from Torchia and Szabo (1982). The twisting correlation function was evaluated at two extremes: in the intermediate zone and the uniform-mode zone using expressions given in Shibata et al. (1985) and Wu et al. (1987). Fourier transforms of these functions were calculated with the aid of techniques described in Allison et al. (1982). Fortran programs to calculate the T_1 values from the various motional models were run on a DEC μVax II.

Results

Deuterium NMR of Partially Hydrated DNA. Room-temperature solid-state ^{2}H NMR spectra and spin–lattice relaxation times (T_1) of both [U-$^2H_{12}$](CGCGAATTCGCG)$_2$ and [S-$^2H_2$](CGCGAAT*TCGCG)$_2$ were obtained after lyophilization and after hydration at 66% relative humidity. Under these conditions, the double-helical oligonucleotide is only partially hydrated and is expected to be in the A form (Falk et al., 1962, 1963a,b; Saenger et al., 1986). In both instances, the corresponding spectra were very similar, and only the line shapes of the samples at the higher hydration level are shown in Figure 1.

The spectrum of the purine-labeled oligonucleotide hydrated at 66% relative humidity is shown in Figure 1a. It can be simulated with an effective asymmetry parameter $\eta_{eff} = 0.07 \pm 0.01$ and an effective deuteron quadrupole coupling constant $(e^2qQ/h)_{eff} = 177 \pm 2$ kHz, assuming no motion. The T_1 value of this sample was found to be ~780 ms however, indicating that there must be rapid, small-amplitude motion of the purine bases in the oligonucleotide to account for the efficient spin–lattice relaxation. Moreover, the coupling constant is slightly reduced from the concensus of values reported by Shindo et al. (1987) and Tsang et al. (1987) of 179 ± 2 kHz for 8-deuteriated adenosine and guanosine nucleosides, again indicating the presence of a rapid libration of the bases. If the values of the quadrupole coupling constant and the asymmetry parameter ($\eta = 0.06 \pm 0.01$) used by Shindo et al. (1987) and Brandes et al. (1986) are assumed to be the static values, then the spectrum of [U-$^2H_{12}$](CGCGAATTCGCG)$_2$ (Figure 1a) can be adequately simulated by assuming that the purine base undergoes a rapid 2-fold libration between two sites that are

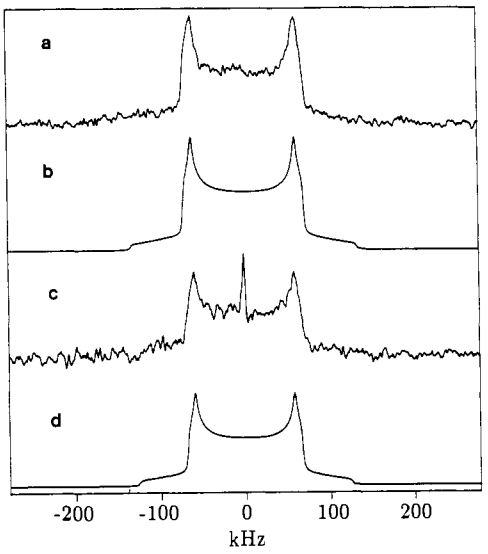

FIGURE 1: Calculated and experimental solid-state ^2H NMR spectra of the uniformly purine-labeled and selectively thymidine-labeled DNA dodecanucleotides hydrated at 66% relative humidity. Spectra were acquired at 298 K at a ^2H NMR resonance frequency of 76.76 MHz with a quadrupole echo pulse sequence. The delay between pulses was 50 μs. Other typical spectrometer settings were as described in the text. (a) Spectrum of [U-$^2H_{12}$](CGCGAATTCGCG)$_2$. A 40-mg sample required 4000 scans with a 3-s recycle delay. (b) Simulation of (a) based on a ±6° librational motion described in the text. The calculated spectrum used $\eta_{static} = 0.06$ and $(e^2qQ/h)_{static} = 179$ kHz. The correlation time of the motion was in the extreme narrowing limit, $\tau_c = 85$ ps. (c) Spectrum of [S-$^2H_2$](CGCGAAT*TCGCG)$_2$. A 30-mg sample required 30 000 scans with a 2-s recycle delay. (d) Calculated spectrum based on a ±10° librational motion, $\eta_{static} = 0.03$, and $(e^2qQ/h)_{static} = 179$ kHz with a correlation time in the extreme narrowing limit.

±6° from the average position (Figure 1b).

The spectrum of the thymidine-labeled oligonucleotide hydrated at 66% relative humidity is shown in Figure 1c. The rigid-lattice simulations yield the values $\eta_{eff} = 0.07$ and $(e^2qQ/h)_{eff} = 168$ kHz. Again, the static model provides an excellent fit to the powder pattern. However, the deuterium spin–lattice relaxation is again quite efficient ($T_1 = 1.6$ s), and the quadrupole coupling constant is significantly smaller than that reported for the nucleoside, $(e^2qQ/h)_{static} = 179 \pm 2$ kHz (Kintanar et al., 1988). These observations indicate the presence of rapid, small-amplitude motion of the thymidine base in DNA. By use of the values for the static quadrupole coupling constant and asymmetry parameter ($\eta_{static} = 0.03$) reported by Kintanar et al. (1988), the spectrum of [S-$^2H_2$](CGCGAAT*TCGCG)$_2$ can be adequately simulated by assuming that the thymidine base undergoes a similar libration as the purine bases except with a slightly larger amplitude (±10°) as shown in Figure 1d.

Deuterium NMR of Highly Hydrated DNA. Both deuteriated DNA samples were investigated at higher hydration levels by equilibration at 80% and 87% relative humidity. Under these conditions, all the "first layer" hydration sites on the oligonucleotide are filled, and the duplex DNA is expected to be in the B form (Falk et al., 1962, 1963a,b; Saenger et al., 1986).

The ^2H NMR spectra of [U-$^2H_{12}$](CGCGAATTCGCG)$_2$ as a function of hydration (66%, 80%, and 87% relative humidity) are shown in Figure 2. First, note that there is no significant decrease in the breadth of the powder pattern as a function of hydration. This strongly indicates that there is no large-amplitude motion that is fast ($\tau_c \leq 10^{-8}$ s) on the ^2H NMR time scale. Any such motion would result in a significant narrowing of the line shape because of averaging of the electric field gradient tensor (see Figure 5 and text below).

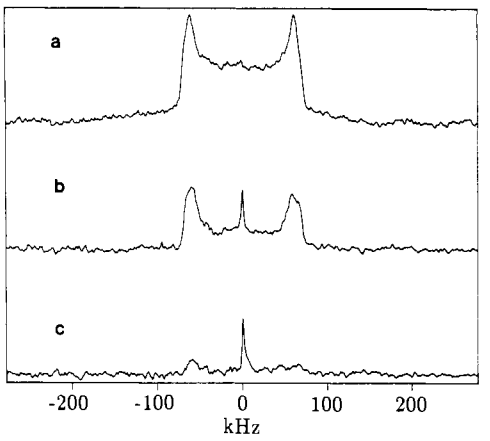

FIGURE 2: Solid-state ^2H NMR spectra of [U-$^2H_{12}$]-(CGCGAATTCGCG)$_2$ hydrated at different relative humidities: (a) 66%, (b) 80%, and (c) 87%. All three spectra were acquired at 298 K with 8000 scans, 50-μs pulse delay, and a recycle delay of $\sim 5T_1$ to ensure thermal (spin) equilibrium and are plotted at the same absolute intensity. Other typical spectrometer settings were as described in the text.

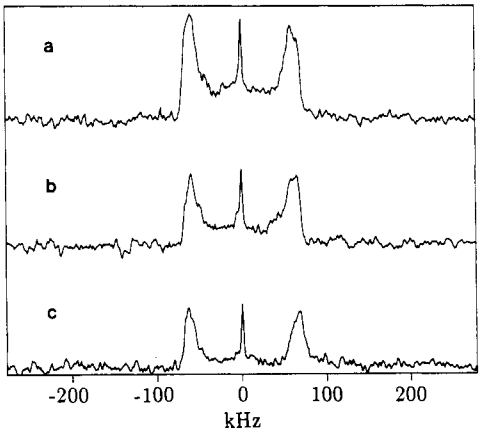

FIGURE 3: Solid-state ^2H NMR spectra of [U-$^2H_{12}$]-(CGCGAATTCGCG)$_2$ hydrated at 80% relative humidity. The spectra were acquired at 298 K with 8000 scans and a 1-s recycle delay with three different pulse delays: (a) 50, (b) 70, and (c) 90 μs.

Second, note the decrease in spectral intensity of the sample with increasing hydration level. All three spectra shown in Figure 2 were scaled to the same absolute intensity and plotted on the same vertical scale. Part of the intensity loss may be attributed to back-exchange of deuterons at the 8-position with H_2O. Solution ^1H NMR spectroscopy of this sample after the solid-state ^2H NMR study was completed indicated a loss of $\sim$30% of the deuterium label, primarily from the guanosine residues. However, back-exchange cannot by any means account for all of the effect. Moreover, a similar effect (data not shown) was observed for [S-$^2H_2$](CGCGAAT*TCGCG)$_2$, in which no loss of deuterium label occurred over the entire course of the study. Therefore, the loss in NMR signal at high hydration levels signifies a decrease in T_{2e}, the so-called echo spin–spin relaxation time, which reflects a reduction of the magnetization at the time of the refocusing of the echo.

The dramatic reduction in signal is undoubtedly due to slow motion that occurs during the formation of the quadrupole echo (Spiess & Sillescu, 1981). Such a motion can cause the quadrupolar dephasing frequency of a given spin to be different from the refocusing frequency, so that no quadrupole echo occurs for that particular spin. Moreover, in order for such a process to be effective, the motion must occur with a cor-

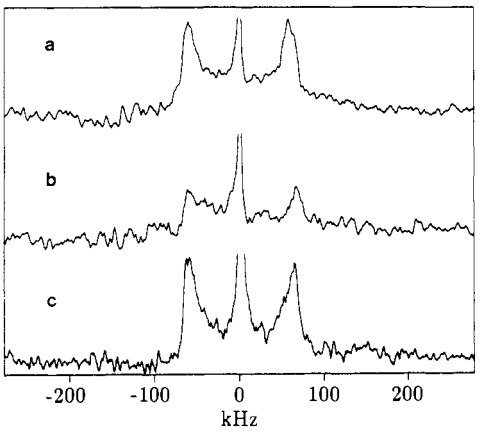

FIGURE 4: NMR spectra of $[S\text{-}^2H_2](CGCGAAT^*TCGCG)_2$ and $[U\text{-}^2H_{12}](CGCGAATTCGCG)_2$ at high hydration levels. The spectra were acquired at 298 K with a 500-ms recycle delay and a 50-μs pulse delay. (a) $[S\text{-}^2H_2](CGCGAAT^*TCGCG)_2$ hydrated at 80% relative humidity, 40 000 scans. (b) Same as in (a) except 87% relative humidity, 200 000 scans. (c) $[U\text{-}^2H_{12}](CGCGAATTCGCG)_2$ hydrated at 87% relative humidity, 120 000 scans.

relation time between 10^{-4} and 10^{-6} s.

Further evidence for slow motion in highly hydrated DNA is shown in Figure 3. The ^2H NMR spectra of $[U\text{-}^2H_{12}]$-$(CGCGAATTCGCG)_2$ hydrated at 80% relative humidity are shown as a function of the delay between pulses in the quadrupole echo pulse sequence. There is a significant change in the line shape, notably a decrease in intensity at the center of the powder pattern, as the pulse delay is increased from 50 to 90 μs. This behavior is due to an anisotropic T_{2e} which arises because the anisotropic slow motion affects some orientations of the C–D bond vector more significantly than others. In the case of $[U\text{-}^2H_{12}](CGCGAATTCGCG)_2$ hydrated at 66% relative humidity, there was little change in the shape of the powder pattern as the pulse delay was varied over the same interval (spectra not shown). This confirms the absence of any significant slow motion in the partially hydrated DNA.

Figure 4 shows the ^2H NMR spectra of $[S\text{-}^2H_2]$-$(CGCGAAT^*TCGCG)_2$ hydrated at 80% and 87% relative humidity and of $[U\text{-}^2H_{12}](CGCGAATTCGCG)_2$ hydrated at 87% relative humidity. As already noted, the spectra of the thymidine-labeled dodecamer show the same intensity loss with increasing hydration as was observed with $[U\text{-}^2H_{12}]$-$(CGCGAATTCGCG)_2$. This is not immediately evident from the spectra shown in Figures 1c and 4a,b because they are not scaled to the same absolute intensity. However, we estimate that for $[S\text{-}^2H_2](CGCGAAT^*TCGCG)_2$ at 87% relative humidity we are observing only 20% of the signal. Note also that the overall line shapes of the two samples at each corresponding hydration level are remarkably similar, although the overall spectral breadth (which is proportional to the quadrupole coupling constant) is less for the $[S\text{-}^2H_2]$-$(CGCGAAT^*TCGCG)_2$ sample.

The spin–lattice relaxation times (T_1) of the purine-deuteriated oligonucleotide were also measured and were found to decrease dramatically upon going from partial hydration (lyophilized powder, 66% relative humidity) to high levels of hydration (80% and 87% relative humidity). The T_1 values of $[U\text{-}^2H_{12}](CGCGAATTCGCG)_2$ as a function of increasing hydration were 1.66, 0.78, 0.24, and 0.18 s, respectively. The sharp decrease in the T_1 parallels the loss in quadrupole echo signal intensity. The T_1 values of the thymidine-labeled dodecamer were also measured at three hydration levels (lyophilized powder and 66% and 80% relative humidity) and were found to be 2.46, 1.56, and 0.36 s as a function of increasing hydration.

Discussion

Analysis of the NMR Line Shapes. The ^2H NMR line shapes shown in Figures 2–4 provide important insights into the nature of the motion in the highly hydrated duplex dodecamer DNA. The strategy adopted here is to consider several possible models for the dynamics of DNA and to observe their effects on the calculated ^2H NMR spectrum. The simulated line shapes are then evaluated as to how well they reproduce the features of the experimental spectra in Figures 2–4.

The goal is not to obtain an exact fit to the actual data. Clearly, if enough parameters are put into a given model, it is possible to simulate any kind of line shape. However, whether such a model would be unique or even physically meaningful would be doubtful at best. Therefore, the aim is to evaluate simple models containing only a few motions that can qualitatively account for the spectral features and trends observed. Whenever possible, models of motion that do not adequately reproduce the data will be discounted.

First, a model containing only a single fast motion ($\tau_c \leq 10^{-8}$ s) is considered. As stated earlier, this model, in which the motion is of small amplitude, can readily account for the ^2H NMR line shapes of $[U\text{-}^2H_{12}](CGCGAATTCGCG)_2$ and $[S\text{-}^2H_2](CGCGAAT^*TCGCG)_2$ at low levels of hydration when the duplex DNA dodecamers are presumably in the A form (Figure 1). We have chosen a model of simple 2-fold jumps for the rapid small-amplitude motion although almost any motional model will work as well, as long as the motion is of small amplitude. For example, Shindo et al. (1987) and Brandes et al. (1986) favor a model which allows two perpendicular directions for the fast libration. Their simulations and spectra are very similar to those shown in Figure 1, and the amplitudes of the fast motion they propose for the partially hydrated DNA are quite similar to those indicated in the present study (~6°–10°). However, any model containing only rapid ($\tau_c \leq 10^{-8}$ s) small-amplitude motions cannot account for the loss of quadrupole echo intensity and the change in line shape with increasing pulse decay that is observed at higher hydration levels. These observations dictate the presence of another slower motion.

Also, it has been proposed by those investigating NMR relaxation times of B-DNA in solution that there is large-amplitude local internal angular motion of the oligonucleotide bases (Hogan & Jardetzky, 1980; Clore & Gronenborn, 1984; Kearns, 1987) occurring with correlation times of ~1 ns. The effect of such motions on the solid-state ^2H NMR line shapes is considered in Figure 5. Figure 5a shows the same simulation as in Figure 1b, corresponding to a purine C8 deuteron undergoing rapid ($\tau_c \sim 1$ ns), small-amplitude (±6°) 2-fold jumps. It is shown for purposes of comparison. Figure 5b shows the calculated spectrum of a base deuteron undergoing jumps between two sites ±20° about the equilibrium position with $\tau_c = 10^{-9}$ s; and Figure 5c simulates nearest-neighbor jumps among six sites evenly distributed on a cone of semiangle 20° also with a 1-ns correlation time. Clearly, the last two calculated powder patterns (Figure 5b,c) are very different from the experimental line shapes. They contain features which are much narrower than anything observed experimentally. The differences become more significant when even larger amplitude motions are considered. Thus, the conclusion is that there are no large-amplitude motions of the base occurring on a nanosecond time scale in the solid hydrated oligonucleotide, even under conditions of high relative humidity

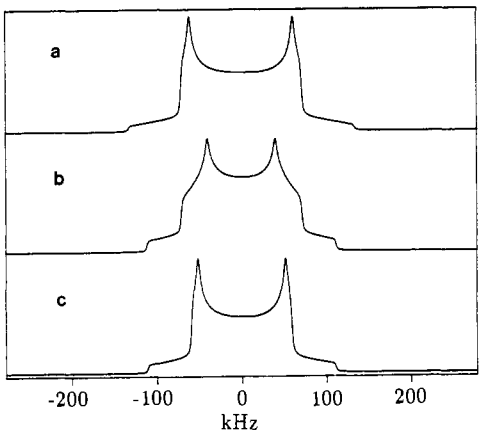

FIGURE 5: Calculated spectra showing the effect of various fast ($\tau_c \leq 1$ ns) motions on the ^2H NMR powder pattern line shape. The static parameters used were the same as for Figure 1b. (a) Same as Figure 1b (6° 2-fold libration) shown for comparison. (b) Same as in (a) but with a jump amplitude of ±20°. (c) Calculated spectrum based on a model of jumps between six sites evenly distributed on a cone of semiangle 20°. This model approximates the "diffusion-in-cone model".

when the DNA is in the same helical form (B form) as in solution.

The experimental spectra of [U-$^2H_{12}$]-(CGCGAATTCGCG)$_2$ hydrated at 80% relative humidity can be accounted for more successfully by a model involving rapid ($\tau_c \leq 10^{-8}$ s) small-amplitude 2-fold jumps and slow ($\tau_c \sim 10^{-4}$ s) small-amplitude 2-fold jumps. In this model, the amplitude of the fast libration was fixed at ±9°, and both the rate and amplitude of the slow motion were varied. In addition, two directions of the slow motion were considered, corresponding to motion about the helix axis and tilts or bends of the helix axis. In general, for both directions of the slow motion, the model could simulate the overall loss in quadrupole echo intensity and the increased center dip as a function of pulse delay quite well with only small-amplitude jumps (~ ±5°). The optimum correlation time appeared to be 50 μs. When τ_c was 5 or 10 μs, the center dip was too deep and the overall intensity was too low. On the other hand, a correlation time of 1 μs accounted for these features quite well, but the two directions of slow motion could no longer be distinguished in the calculated spectra. In addition, for the shorter correlation time, the simulated line shapes (not shown) did not fit as well as the calculated spectra with $\tau_c \sim 50$ μs. Examples of the calculated line shapes with 50-μs correlation time for two pulse delays are shown in Figure 6a–d. These should be compared directly to Figure 3a,c.

It should be noted at this point that all simulations did not include the effects of a finite T_2 value which could arise from ^1H–^2H dipolar coupling. Assuming a reasonable T_2 value of 300 μs (which can be estimated from the intensity loss of partially hydrated DNA as a function of pulse delay, e.g., at 66% relative humidity), an additional intensity loss of ~20% on going from 50- to 90-μs pulse delay may be expected. Inclusion of this effect generally improves the fit of the simulations but does not require a modification of any of the motional models under consideration.

While the simple model of fast and slow small-amplitude motions about two axes can account for many features of the experimental data, there are still minor problems with the line shapes. In particular, for the case when the slow motion is about the helix axis, the falloff from the horns to the outside of the pattern is too vertical especially at the longer pulse delay (Figure 6a,b). On the other hand, when the slow motion

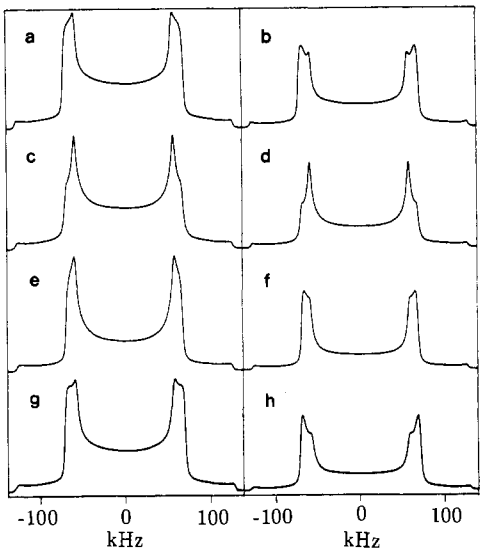

FIGURE 6: Calculated spectra showing the effect of various slow ($\tau_c \sim 10$–100 μs) motions on the ^2H NMR powder pattern line shape. Each model included a fast small-amplitude libration (9°, $\tau_c \sim 125$ ps) and used $\eta = 0.06$ and $e^2qQ/h = 179$ kHz. Simulated spectra were calculated for two values of the pulse delay in the quadrupole echo pulse sequence (50 and 90 μs). (a and b) Slow libration parallel to the helix axis with amplitude ±5° and $\tau_c = 50$ μs. (c and d) Same as in (a) and (b) but slow libration perpendicular to the helix axis. (e and f) Nearest-neighbor jumps among six sites about the helix axis. Each site was separated by 5° of arc, and $\tau_c = 50$ μs. (g and h) All-site jumps between four sites that are separated by ±9° about the helix axis and ±2° about an axis perpendicular to the helix axis with $\tau_c = 125$ μs.

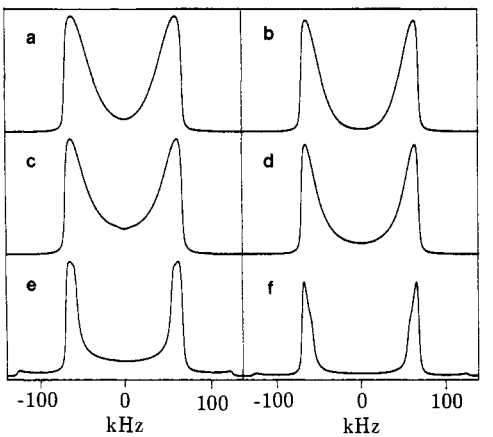

FIGURE 7: Calculated spectra showing the effect of additional models of the slow motion on the ^2H NMR powder pattern line shape. All parameters were the same as for Figure 6 except where noted. (a and b) Nearest-neighbor jumps among six sites about the helix axis. The sites were separated by 30° of arc, and $\tau_c = 170$ ns. No fast libration was included in the simulation. (c and d) Same as in (a) and (b) except the sites were separated by 20° and $\tau_c = 200$ ns. (e and f) With the model as described in Figure 6g,h except $\tau_c = 50$ μs.

consists of tilts of the helix axis, the horns of the simulated powder pattern are too sharp, and there is an inflection point on the outer edge of the horns (Figure 6c,d).

Shindo et al. (1987) have studied much larger DNA molecules using the solid-state ^2H NMR technique. They also observed a significant dependence of the line shape on the pulse delay when the DNA was highly hydrated, similar to the results shown in Figure 3. These workers chose to model the slow motion as six-site nearest-neighbor jumps about the helix axis with an angle of about 30° between sites and a correlation time $6\tau_c \sim 10^{-6}$ s. Calculated spectra based on this model

are shown in Figure 7a,b. Clearly, this model does not do a good job of fitting the line shapes of [U-$^2H_{12}$]-(CGCGAATTCGCG)$_2$ equilibrated at 80% relative humidity. The Shindo model with large-amplitude jumps seems to have more success fitting the line shapes of DNA hydrated at 87% relative humidity as discussed below (Figure 7a–d).

The Shindo model does a fair job of fitting the spectra of [U-$^2H_{12}$](CGCGAATTCGCG)$_2$ hydrated at 80% relative humidity (Figure 3) if the amplitude of the jumps is reduced to 5° and the correlation time is increased to 50 μs. Calculated spectra at two pulse delays are shown in Figure 6e,f. These should be compared to Figure 3a,c. Note that the shape of the horns is similar to the experimental line shapes and the center dip is also reasonably well represented. However, there appears to be too much intensity in the center of the spectrum when the pulse delay is 90 μs (Figures 3c and 6f).

At present, it is unclear how much attention should be paid to the fine details of the experimental line shapes. Because of the paucity of labels per molecule, the small amount of sample, the inherent low NMR sensitivity of the deuteron, and the loss of echo intensity, the signal-to-noise of the spectra is not optimal for detailed line shape simulation. Also, as mentioned earlier it is possible to account for the detailed features of the line shape simply by adding more parameters to the model such as a distribution of quadrupole coupling constants, a distribution of amplitudes of motion, a distribution of rates, etc. Such an approach may not be physically reasonable and will certainly not give a unique answer.

Nevertheless, a number of more complex motional models were considered in trying to fit the line shapes. These models consisted mainly of allowing two orthogonal directions for the slow motion and varying the amplitudes of the motion, the number of sites, and the manner in which the sites are visited (i.e., nearest-neighbor jumps, all-site jumps, etc.). In all cases, only small-amplitude slow motions were required to account for the echo intensity loss and the change in the powder patterns with pulse delay, while at the same time giving reasonable (but not exact) fits to the details of experimental line shapes of the oligonucleotides hydrated at 80% relative humidity. An example of one of these models is shown in Figure 6g,h which should be compared to Figure 3a,c. The simulations in Figure 6g,h included a fast 2-fold small-amplitude libration (±9°, $\tau_c \leq 1$ ns) and a slow ($\tau_c \sim 125$ μs) all-site jump between four positions defined by a ±9° jump about the helix axis and a ±2° tilt of the helix axis.

At this point it should be mentioned that similar models as used to generate Figure 6 (fast, small-amplitude libration of the base plus slow, small-amplitude torsional and/or tilt motion of the helix axis) can be used to calculate spectra (not shown) that can fit the ^2H NMR spectrum of [S-$^2H_2$]-(CGCGAAT*TCGCG)$_2$ hydrated at 80% relative humidity (Figure 4a). It appears that even smaller amplitudes of the slow motion (2°–6°) are required to fit this experimental powder pattern.

This type of motional model may also be adapted to account for the experimental spectra of oligonucleotides at even higher hydration levels (Figure 4b,c). These spectra are characterized by increased echo intensity loss and a deeper center dip at a given pulse delay. Also, the horns of the powder pattern appear to straighten up (i.e., falloff more vertically to the outside), but the certainty of this result is diluted by the poor signal-to-noise of the experimental line shapes. Nevertheless, these features can be accommodated by the model simply by slightly increasing the rate and/or the amplitude of the slow motion. An example of such a calculated spectrum as a function of pulse delay is shown in Figure 7e,f. This simulation is based on the same model used to generate Figure 6g,h except the correlation time was decreased to 50 μs. Note, however, that there are some problems with this line shape. The shape of the center dip seems to be too rectangular and is a little too deep (compare Figures 4c and 7e). Moreover, this model does not adequately account for the overall loss of spectral intensity that was observed on going from 80% to 87% relative humidity, even taking into account that some loss of deuterium incorporation occurred.

The Shindo model with large-amplitude jumps (Figure 7a–d) seems to do a reasonable job with the data obtained at the highest hydration level (Figure 4b,c), although a slightly smaller amplitude than originally proposed by these workers seems to fit the data better (Figure 7c,d). Note especially that the shape of the horns is reasonably well simulated (although they are perhaps a little too broad in the calculated spectra) and the shape of the central dip is no longer rectangular as in the previous model. Note also that the calculated spectrum has no shoulders at the edges of the pattern. Unfortunately, the signal-to-noise of the experimental line shapes obtained at 87% relative humidity is not sufficient to determine the presence or absence of such features. The observation of shoulders in the spectra would tend to argue against the Shindo model with large amplitude jumps. The model also has problems accounting for the loss in spectral intensity as a function of pulse delay at very high hydration levels. Although no such data were obtained (for 87% relative humidity) in the present study, calculated spectra based on the Shindo model (Figure 7a–d) did not appear to fit the data of Shindo et al. (1987) shown in their own Figure 3. In particular, there was not enough overall spectral intensity loss in the simulated spectrum at the longer pulse delay (Figure 7b,d).

In summary, models which include slow ($\tau_c \sim 10^{-4}$–10^{-6} s) motions of varying amplitudes are sufficient to account for the observed loss of spectral intensity at high hydration levels as well as, to a large extent, the change in line shape as a function of pulse delay, especially at 80% relative humidity. However, the overall model, which also includes a rapid ($\tau_c \leq 10^{-8}$ s) small-amplitude libration, cannot fit the fine details of the line shapes obtained at high hydration levels. This suggests the presence of more complex motions which may include a distribution of amplitudes and correlation times. Such a phenomenon could arise from collective torsional fluctuations as discussed below.

Spin–Lattice Relaxation in Hydrated DNA. The results of the present investigation are similar to those of previous ^2H NMR studies (Brandes et al., 1986; Shindo et al., 1987). The fast small-amplitude libration observed in all three investigations under all hydration conditions is probably a true local, internal, angular motion of the bases. In fact, this motion is probably quite similar to the fastest motion observed by fluorescence depolarization anisotropy studies of ethidium bromide intercalated into DNA, which had been attributed to wobbling of the dye in its binding site (Magde et al., 1983; Shibata et al., 1985).

We can estimate the rate of the fast libration if we assume that it is the only motion contributing to spin–lattice relaxation. This seems quite reasonable up to a hydration level of 66% relative humidity, since no other significant motion shows up from the line-shape analysis. If the amplitude of the jump is assumed to be 6° as indicated by the simulated spectrum, then a correlation time of 85 ps is required to account for the observed T_1 of 780 ms for [U-$^2H_{12}$](CGCGAATTCGCG)$_2$ hydrated at 66% relative humidity. This was calculated on

the basis of the 2-fold jump model of Torchia and Szabo (1982) where we have ignored the effects of the nonzero asymmetry parameter. The longer T_1 value of the lyophilized sample can be accommodated simply by slightly reducing the amplitude of the motion and/or slightly increasing the rate. Alternatively, our original assumption may be in error and additional fast motions are contributing to spin–lattice relaxation, but these are of such small amplitude that they have a negligible effect on the line shapes.

The T_1 values of partially hydrated [S-$^2H_2$]-(CGCGAAT*TCGCG)$_2$ are higher than those of the purine-labeled dodecamer at the corresponding hydration level, despite the fact that the line-shape analysis seems to indicate a larger amplitude fast libration of the thymidine compared to either of the purines. Whether or not this latter effect is real depends on the accuracy of the static values of the quadrupole coupling constant reported in the literature (Tsang et al., 1987; Kintanar et al., 1988). These values were determined from the appropriately labeled nucleoside monomers, and they could easily be in error by as much as 2% if internal motion in the model compound was not properly taken into account. Thus, if the $(e^2qQ/h)_{static}$ value for the purines is slightly underestimated and/or if it is slightly overestimated for 6-deuteriated thymidine, then the apparent difference in the amplitudes of the fast libration could be insignificant. In any case, the T_1 data for the deuteriated DNA samples imply a faster libration for the thymidine base compared to the purines at a given hydration level. This observation, together with the possibility of larger amplitude fast motion of the thymidine (as compared to the purines), suggests that there may be a propeller-twisting component to the rapid libration of the base pairs.

The amplitude of the fast libration appears to increase at a hydration level of 80% relative humidity or higher, giving rise to a small but significant decrease in the overall spectral breadth. Contrary to the suggestion of Shindo et al. (1987) and Brandes et al. (1986), it does not appear necessary to increase the amplitude of the fast libration on going to even higher hydration levels. The effective quadrupole coupling constant (or alternatively the overall breadth of the powder pattern at half-height), which is the parameter most affected by the fast libration, does not change significantly on going from 80% to 87% relative humidity for the DNA samples investigated here. However, the quadrupole splitting measured at the singularity does appear to be more sensitive to the hydration level. This implies that the nature of the motion may be changing but not necessarily the amplitude.

The increased amplitude of the fast libration on going from 66% to 80% relative humidity is expected to have a significant effect on the observed ^2H spin–lattice relaxation time. If the rate of the motion is assumed to remain the same, then the larger librational amplitude can account for a significant portion of the observed sharply decreased T_1 value at 80% relative humidity. Thus, for a correlation time of $\sim$85 ps, an increase in amplitude from 6° to 9° results in a decrease in the T_1 value from 780 to $\sim$350 ms. The additional difference of the calculated and observed T_1 of [U-$^2H_{12}$]-(CGCGAATTCGCG)$_2$ hydrated at 80% relative humidity (350 vs 240 ms) must be accounted for by increasing the correlation time (τ_c) of the libration or including the contribution of other motions of the DNA to spin–lattice relaxation. Similar reasoning may be used to account for the further decrease in the T_1 value at 87% relative humidity.

A slow motion was observed to occur in DNA at high hydration levels in this and previous ^2H NMR studies (Shindo et al., 1987; Brandes et al., 1986). It is more difficult to accurately depict the nature of the slow motion because of the problems obtaining a unique fit of the simulations to the experimental line shapes especially at the highest hydration level investigated.

For the hydration level obtained by equilibrating at 80% relative humidity, it appears that the slow motion may be quite reasonably modeled as restricted jumps about the helix axis. It does not seem to matter whether there are two or six equatorial sites as long as the jumps are of small amplitude ($\sim$5°). The addition of small-amplitude jumps in an orthogonal direction (tilts of the helix axis) has only a small effect on the calculated spectra and need not be included in the analysis of spin–lattice relaxation. The optimum rate for the jumps about the helix axis appears to be 10 kHz. We evaluated this slow motion as a possible contributor to the effective spin–lattice relaxation at high hydration levels. Rough calculations show that this motion is much too slow and of much too small an amplitude to make any significant contribution.

At the highest hydration level (87% relative humidity), none of the models of the slow motion that were considered could adequately reproduce all the experimental features. Evidently, more complex models for the motion must be considered (including possibly several fast, small-amplitude motions); but the difficulties in obtaining high-quality ^2H NMR data in highly hydrated DNA prevents such an undertaking at the present time. However, even surmounting this obstacle may not be enough because it may not be possible to determine a unique model for the motion. Nevertheless, the data indicate there must be a significant motional component occurring at 10–100 kHz in order to account for the severe quadrupole echo intensity loss at high hydration level. This slow motional component may have a significant angular amplitude. Again, the slow motion was evaluated as a possible contributor to spin–lattice relaxation. However, calculations show that, despite the possible large amplitude of these motions, the rate is too slow to make any significant contribution greater than a few percent.

Note that while the slow motion has been modeled as restricted jumps about the helix axis, this may not be a physically realistic model. An alternative model is very slow Brownian rotation about the helix axis with no restrictions. However, since the line shapes are most sensitive to motions occurring on a time scale of $\sim$10 μs, this time may be too short for complete rotational diffusion to occur, and so the motion appears to be restricted. It is also worth noting at this time that since the oligonucleotide has a rather low aspect ratio, the time scale for tilting motions of the helix axis will not be significantly slower than rotation about this axis.

Collective Torsional Fluctuations in Hydrated DNA. It is worthwhile to consider the results of this study within the context of a dynamical theory that treats DNA as a deformable filament (Barkley & Zimm, 1979; Allison & Schurr, 1979; Schurr, 1984) which can both bend and twist. The DNA is considered to be a linear array of $N + 1$ identical rods connected by Hookean springs which oppose the twisting and bending deformations of the macromolecule. The dynamics of the system is contained in the correlation function which is considered to be sums of the product of bending (tumbling), twisting, and internal correlation functions.

In short DNA oligonucleotides such as those used in the present study, bending is expected to be of very small amplitude and may be considered negligible. Moreover, as the DNA samples are in a solid phase there is negligible end-

over-end tumbling of the duplex on the time scale of the NMR experiment, even at high hydration levels. Therefore, the bending correlation function will be ignored ($F_a = 1.0$). The twisting correlation function must be divided into several time zones which exhibit different types of decay. The demarcation of the time zones depends on the length of the DNA as well as the torsional friction factor (γ) of a subunit rod and the torsional force constant (α) between the rods. Schurr and co-workers have derived accurate formulas for the twisting correlation function (C_a) in all the time zones (Shibata et al., 1985; Wu et al., 1987). Finally, the internal correlation function (I_a) may be obtained from the work of Torchia and Szabo (1982) for simple models of internal motion (e.g., 2-fold jumps).

By use of the zone limits defined in Wu et al. (1987) and reasonable values for α (4×10^{-12} dyn·cm) and γ (6×10^{-23} dyn·cm·s) determined by fluorescence depolarization studies of DNA in solution, the preuniform-mode zone and uniform-mode zone are predicted to begin at, respectively, 30 ps and 1.5 ns for a duplex dodecamer. However, the friction factor (γ) is directly proportional to the viscosity (η), which is very much higher in our hydrated solid samples. Therefore, the different modes of the torsional motion are expected to occur at much later times. In fact, the slow motion observed in this study, which we have modeled primarily as motion about the helix axis occurring on a time scale of 10–100 μs, is probably the uniform mode (i.e., motion of the entire dodecamer about the helix axis). And higher mode zones, with correlation functions that are no longer simple exponential functions of time, are also shifted to longer times than expected in solution, and some modes may occur on a nanosecond time scale.

The root-mean-square amplitudes of motion of all these torsion normal modes are all expected to be quite small. These values can be estimated from the residual amplitude of the twisting correlation function at times long enough to be in the uniform-mode zone (Shibata et al., 1985). For an oligonucleotide of 12 base pairs and for the value of the force constant (α) given above, the amplitude of the torsional motion is expected to be $\sim\pm 9°$. Indeed, no evidence for large-amplitude nanosecond motion is found in our ^{2}H NMR spectra under all conditions studied. In addition, it would be difficult to differentiate the rapid small-amplitude local internal motion we observe from the higher (collective) torsional modes because all are occurring in the rapid limit ($\tau_c \leq 10^{-9}$ s) of the ^{2}H NMR time scale and the line shapes are less sensitive to the details of the motion in this temporal regime. Moreover, the collective torsional modes appear to become more important when the DNA is more highly hydrated and the viscosity is no longer essentially infinite. Unfortunately, this parallels the degradation in the signal-to-noise of the spectra by the slow motion. Nevertheless, the small-amplitude rapid collective torsional motions may be responsible for some of the line-shape changes observed at the higher hydration levels, which we were unable to account for by considering simple models for the slow motion.

The spin–lattice relaxation time (T_1) is probably affected quite significantly by the rapid collective torsional motions if they indeed occur on the nanosecond time scale as we have proposed here. Thus, the sharp decrease in the T_1 value that was observed on going from 66% relative humidity to higher hydration levels may be explained by the onset of collective torsional motions occurring at rates where the amplitudes of the spectral density are near the maximum. It is not necessary to propose that the local, internal libration of the bases increases in amplitude or, more importantly, that the rate of the local libration slows down. The contribution of the higher mode zones to spin–lattice relaxation has been estimated from the expression for the intermediate-zone twisting correlation function. This is a rather crude approximation since this formula is only valid for long DNA molecules and does not include end effects which undoubtedly are quite significant in our DNA samples. Nevertheless, these calculations show that higher mode collective torsional fluctuations contribute significantly to spin–lattice relaxation and can easily account for the decreased T_1 values we observe at high hydration levels.

It should be noted that both Shindo et al. (1987) and Brandes et al. (1986) measured spin–lattice relaxation times for their respective DNA samples at two fields (corresponding to ^{2}H NMR resonance frequencies of 38.4 and 76.8 MHz). Both groups found that the ratio of the values at the two fields [$T_1(76.8)/T_1(38.4)$] was between 2 and 3 for highly hydrated DNA. This result is not compatible with a motional model consisting of a single rapid libration occurring with a correlation time on the so-called fast side of the T_1 curve ($\tau_c \leq 1$ ns). Moreover, if the libration is occurring with a correlation time on the slow side of the T_1 curve, the theoretical value of $T_1(76.8)/T_1(38.4)$ should be 4. Brandes et al. (1986) correctly point out that this anomaly can be accounted for by including collective torsional modes, which necessitate a distribution of correlation times. This arises directly from the form of the twisting correlation function, which, in addition to spreading over several time zones encompassing different normal modes, is in general no longer a simple exponential function of time (Shibata et al., 1985; Wu et al., 1987).

Comparison of DNA in Solution and Solid Phases. The view of the dynamics that is emerging from solid-state ^{2}H NMR studies of hydrated DNA seems to be largely in agreement with the results determined from fluorescence depolarization when analyzed within a theoretical framework that treats DNA as a deformable filament. The difference is that the friction factor (γ) is very much higher in the solid phase than in solution, as is to be expected from the different viscosities of the two media. Thus, the limits of the different torsional normal modes are shifted to longer time.

At present, the effect of hydration on the torsional force constant (α) is unknown. However, this parameter represents the potential for twisting between two base pairs and presumably depends closely on the local structure. Therefore, it is not unreasonable for the force constant to change with hydration because the helical structure can also change (e.g., from A form to B form) with increasing hydration. Falk et al. (1962, 1963a,b) have studied the hydration of DNA using several techniques. They find that at 80% relative humidity all hydration sites of the DNA molecule are filled and the helix has the B-form structure. Above this hydration level, water fills the spaces between the DNA molecules, and the sample is observed to swell. The DNA oligonucleotide has adopted the most favorable structure (B form) when the first hydration layer is complete, and further hydration causes little further changes in local structure. Therefore, at 80% relative humidity, the force constant (α) is presumably the same as that observed in solution, and the amplitude of collective torsional deformations (which depends primarily on the force constant) is the same as that in solution. What changes as a function of hydration is the friction factor (γ) and thus the time scale of the torsional fluctuations.

Similar arguments may be made about the nature of internal motions of DNA in solution and in the hydrated solid. The rate and amplitude of the internal motion are determined by the energy potential which in turn must depend primarily on

the local structure. Thus, the internal motions that are observed in highly hydrated B-form DNA are similar to those that occur in solution. The addition of more water in the interstices between DNA molecules is not expected to significantly perturb the energy potential which determines the internal motion. This interpretation contravenes the solution NMR relaxation studies of DNA dynamics which predict large amplitudes of internal motion on a nanosecond time scale. In the present study, we find no evidence whatsoever for any base motion with an amplitude greater than ±10° occurring on such a rapid time scale.

Implications for Investigation of Site-Specific Motion. The present study is the first attempt to determine spectroscopically the dynamics of a defined sequence of DNA as well as the dynamics of a particular site on a defined sequence. However, the results of this study are very similar to the results of previous ^{2}H NMR studies of longer DNA molecules with undefined sequence. In particular, the experimental line shapes derived from this and previous ^{2}H NMR studies indicate that the dynamics are very similar in all the systems studied. There are minor differences in some of the measured spin–lattice relaxation times, but these may be ascribed to the differing lengths of the molecules studied which would affect the twisting correlation function that applies to the relaxation. Another possible source of the dissimilar T_1 values is slight differences in the preparation (e.g., humidification) of the various samples. At any rate, these small discrepancies were not pursued in any detail.

As noted earlier, there appears to be some difference in the rate and amplitude of the fast libration of the labeled thymidine when compared to the purines. Whether this phenomenon is a general property of the pyrimidines or a site-specific effect cannot be determined at this point. The selectively labeled sample showed the same features and trends upon hydration as the uniformly labeled sample.

The current work shows that studies of site-specific motion in DNA are certainly feasible albeit very difficult. If our interpretation is correct, future studies will yield the most information at a hydration level corresponding to 80% relative humidity. At this hydration level, the DNA molecule is fully hydrated and is presumably in the B form. At the same time, the sample has just started to swell so that slow twisting and perhaps tumbling of the entire helix are not too significant and the signal-to-noise ratio of the quadrupole echo experiment is not hopelessly degraded. At 80% relative humidity, all the interesting motion is already taking place, and further hydration only enhances the overall twisting and tumbling of the entire molecule.

Summary and Conclusions. In summary, we have obtained ^{2}H NMR spectra and spin–lattice relaxation times as a function of hydration level of two samples of the Na salt of a defined self-complementary DNA sequence, [d-(CGCGAATTCGCG)]$_2$, deuteriated either at the C8 position of all the purines or at the C6 position of the innermost thymidine (T7) in the sequence. At low levels of hydration (66% relative humidity or less) when the DNA is only partially hydrated, all the ^{2}H NMR spectral data may be explained quite adequately by a motional model involving only a small-amplitude (~6°–10°) 2-fold libration of the bases with a correlation time $\tau_c \leq 85$ ps. This motion is likely a true local internal angular motion of the bases. At higher levels of hydration (80% relative humidity or greater) when the DNA is fully hydrated, there is a dramatic decrease in spectral intensity of the quadrupole echo and a variation of the line shape as a function of the delay between pulses in the quadrupole echo sequence. These results indicate unequivocally that there is significant motion with correlation time $\tau_c \sim 10$ μs. The amplitude of this relatively slow motion is quite small at 80% relative humidity as indicated by simulation of the line shapes, but the amplitude may increase at higher hydration levels. The slow motion is apparently some type of rotation about the helix axis although some component of tilts of the helix axis (tumbling of the entire helix) cannot be ruled out. All the experimental spectra obtained in this study are inconsistent with the presence of rapid ($\tau_c \leq 10$ ns) large-amplitude (>10°) motions of the base. The ^{2}H NMR line shapes of highly hydrated DNA cannot be fully simulated with a motional model consisting only of a fast small-amplitude libration of the bases and a slow motion about the helix axis. More complex models must be considered.

The spin–lattice relaxation times also show a dramatic decrease on going from 66% relative humidity to 80% relative humidity. This result may be accommodated by increasing the amplitude and/or decreasing the rate of the internal motion. The slow motion does not contribute significantly to spin–lattice relaxation. Alternatively, all the data admit to interpretation within a theoretical model that allows collective torsional (and perhaps bending) deformations of the DNA. In this model, the friction factor (γ), which depends on the viscosity, is much greater in the hydrated solid than in solution. Thus the limits of the various zones of the twisting correlation function are shifted to longer times than are observed for DNA in solution. The slow motion observed at high hydration levels is attributed to the uniform twisting mode (torsion of the entire molecule about the helix axis), and perhaps, tumbling of the entire helix must also be considered since the dodecamer oligonucleotide has a very low aspect ratio. The higher order collective torsional modes are also shifted to longer times and probably occur on the nanosecond time scale. These modes contribute to spin–lattice relaxation quite effectively because the spectral densities are near their maximum amplitudes. This can account for the reduction in the T_1 value at high hydration. The amplitudes of the torsional motion remain quite small (thus the collective torsional fluctuations do not dramatically affect the line shapes) because the torsional force constant (α) is presumably not substantially different than the value found for DNA in solution.

Further studies are necessary to clarify the details of the internal and collective torsional motions of DNA. We are pursuing investigations of the dynamics of other moieties of DNA by selectively labeling the 2″ or 5′ and 5″ positions of nucleosides and then incorporating these deuteriated residues into the same DNA sequence studied here. DNA sequences containing deuteriomethyl-labeled thymidine are also currently under investigation. It is hoped that the methyl-labeled samples will yield spectra with higher signal-to-noise which are amenable to more detailed analysis. In addition, we intend to measure spin–lattice relaxation times of longer deuteriated oligonucleotides with defined sequence to investigate the length dependence of the twisting correlation function. Relaxation studies at different field strengths are also planned. Taken together, these studies will provide a more rigorous test of the dynamical models described here.

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APPENDIX

Deuterium is a spin 1 nucleus, and therefore, its NMR spectral properties are dominated by the electric quadrupole interaction. The relaxation rate is given by

$$\frac{1}{T_1} = \frac{\omega_Q^2}{3}[J_1(\omega_0) + 4J_2(2\omega_0)] \quad \text{(A1)}$$

where $\omega_Q = 3e^2qQ/4\hbar$ and the spectral density $J_m(\omega)$ is defined as the Fourier transform of the correlation function $K_m(t)$:

$$J_m(\omega) \equiv 2\mathrm{Re}\int_0^\infty K_m(t)e^{i\omega t}\,dt \quad \text{(A2)}$$

The correlation function is defined by

$$K_m(t) \equiv \langle R^*_{2m}(0)R_{2m}(t)\rangle \quad \text{(A3)}$$

where the R_{2m} are the spherical components of the electric field gradient (EFG) tensor in the laboratory frame. Using Wigner rotation matrices, we express R_{2m} in terms of spherical components of the EFG tensor in the principal axis system (PAS) and obtain

$$K_m(t) = \sum_{a,a'=-2}^{2} \langle D^{*(2)}_{am}(\Omega_{CL}(0))D^{(2)}_{a'm}(\Omega_{CL}(t))\rangle \times \langle D^{*(2)}_{0a}(\Omega_{PC}(0))D^{(2)}_{0a'}(\Omega_{PC}(t))\rangle \quad \text{(A4)}$$

where Ω_{PC} is the set of Euler angles which describes the instantaneous orientation of the PAS frame (the principal component of which lies along the C–D bond) in the subunit (base pair) frame and Ω_{CL} describes the instantaneous orientation of the subunit frame in the laboratory frame. Note that to obtain this last expression we have assumed that the EFG tensor of a deuteron in a C–D bond is axially symmetric.

We wish to calculate the so-called powder average T_1, and so we calculate the spatial average of the correlation function. It can be shown that only the diagonal terms of the sum ($a = a'$) survive the averaging process. Furthermore, the m dependence of the correlation function is removed. Thus

$$K(t) = \frac{1}{5}\sum_{a=-2}^{2} C_a(t)G_{aa}(t) \quad \text{(A5)}$$

The total correlation function is the product of the internal correlation function $[G_{aa}(t)]$, which describes the motion of the C–D bond in the base pair frame, and a correlation function which describes the motion of the base pair subunit in the laboratory. In a more general treatment, the latter function includes contributions of bends and twists of the deformable DNA double helix. However, for reasons described in the text, we neglect the contributions of helix bending (tumbling). Thus, $C_a(t)$ is the twisting correlation function given by Schurr and co-workers (Schurr, 1984; Shibata et al., 1985; Wu et al., 1987). The factor 1/5 is a normalization constant which arises in the derivation of the subunit correlation function (Schurr, 1984; Langowski et al., 1985). Expressions for $G_{aa}(t)$ may be obtained from Torchia and Szabo (1982).

We wish to evaluate spin–lattice relaxation times for two representative temporal zones of the twisting correlation function. The so-called intermediate zone is defined by the limits $\gamma/4\alpha \le t \le \tau_{31}$, where γ is the friction factor ($\sim 6 \times 10^{-23}$ dyn·cm·s in solution), α is the torsion force constant ($\sim 4 \times 10^{-12}$ dyn·cm), and τ_j is given by

$$\tau_j = \frac{\gamma}{4\alpha \sin^2[(j-1)\pi/2(N+1)]} \quad \text{(A6)}$$

N is the number of base pairs. Thus for a DNA dodecamer in solution, the intermediate zone runs from 3.8 to 17 ps. In the hydrated solid, γ which is directly proportional to the viscosity may be quite a bit larger, and consequently, the zone may be shifted to longer times. The uniform-mode zone is defined by the limit $t \ge 6\tau_2$. Again for a dodecamer in solution this zone is expected to begin at 1.6 ns, but it may be shifted to longer times in the hydrated solid DNA.

The twisting correlation function in the uniform-mode zone is given by

$$C_a(t) = B_a(\infty)\exp\left[\frac{-a^2k_BTt}{(N+1)\gamma}\right] \quad \text{(A7)}$$

where

$$B_a(\infty) = (a^2Z)^{-1/2}\exp\left(\frac{-a^2Z}{3}\right)\frac{\pi^{1/2}}{2}\mathrm{erf}[(a^2Z)^{1/2}] \quad \text{(A8)}$$

and $Z \equiv k_BT(N+1)/(4\alpha)$. We model the internal motion as a discrete 2-fold libration and obtain from Torchia and Szabo (1982) the expression

$$G_{aa}(t) = d^{(2)}_{0a}(\beta)d^{(2)}_{0a}(\beta)\Gamma_{aa}(t) \quad \text{(A9)}$$

where the $d^{(2)}_{mn}$ are the reduced Wigner rotation matrix elements (Brink & Satchler, 1962), β is the amplitude of the libration, and

$$\Gamma_{00}(t) = \Gamma_{22}(t) = \Gamma_{-2-2}(t) = 1$$
$$\Gamma_{11}(t) = \Gamma_{-1-1}(t) = e^{-t/\tau} \quad \text{(A10)}$$

Substituting eq A7–A10 into eq A5, we obtain $K(t)$, which can be easily Fourier transformed analytically to give the spectral densities which may then be used to calculate a T_1. The spin–lattice relaxation time depends on the parameters τ_c, the internal correlation time, and τ_{tw} which is defined by

$$\tau_{tw} = \frac{(N+1)\gamma}{a^2k_BT} \quad \text{(A11)}$$

For a DNA dodecamer in solution $\tau_{tw} \sim 19$ ns. Using this value and an arbitrary value for τ_c, we find that the uniform torsional mode makes only a small contribution to spin–lattice relaxation. Note that for even larger values of τ_{tw} which we expect in the hydrated solid the contribution of the uniform torsion to spin–lattice relaxation is even smaller.

Similarly for the intermediate zone, the twisting correlation function is given by

$$C_a(t) = \exp\left(\frac{-a^2k_BT\sqrt{t}}{\sqrt{\pi\alpha\gamma}}\right) \quad \text{(A12)}$$

Again we substitute into eq A5 to obtain $K(t)$. However, the resulting correlation function must be Fourier transformed with the aid of techniques described in Allison et al. (1982) and by use of the convolution theorem for Fourier transforms. The spectral densities are then evaluated numerically, and then the T_1 value may be calculated. Spin–lattice relaxation times calculated in this manner for reasonable values of the parameters described in the text can satisfactorily account for the sharply decreased T_1 values observed at high hydration levels.

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