Deuterium Nuclear Magnetic Resonance of Oriented DNA Fibers

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Solid-state ^{2}H NMR spectra were measured as a function of relative humidity for nonoriented DNA films and oriented DNA fibers deuterated at C8 carbons of the purine bases. The line-shape analyses of the quadrupole echo spectra from oriented DNA fibers demonstrated that the purine base planes of the A form of DNA were tilted by about 70° relative to the helix axis, and that base planes of the B form varied significantly, although they were, on average, perpendicular to the helix axis. These results were generally consistent with the X-ray fiber diffraction studies and the single crystal structures of oligonucleotides. Analyses of ^{2}H relaxation times, T_1 and T_2, showed at 79% RH that the orientation of the base planes fluctuated about an axis normal to the helix axis with a correlation time, $\tau_c=3.6\times10^{-8}$ s and amplitude of 8.5°. The correlation time of the base plane fluctuation was 1.5×10^{-8} s and the amplitude was 13.5° at 92% RH. In addition, at 92% RH, but not at 79% RH, the base planes reoriented slowly, $\tau_c \approx 10^{-6}$ s, about the helix axis, through an rms angle of ca. 30°.

We have recently reported ^{31}P nuclear magnetic resonance (NMR) studies of the structure and dynamics of DNA in the fibers as a function of relative humidity.[1,2] The results were in general agreement with the structure inferred from the X-ray diffraction studies of the fibers at relatively low humidities, but differed from the X-ray results, in some respects, at high humidities. The most interesting conclusion derived from the NMR studies is the finding of heterogeneity in the structure and dynamics in the backbone of the B form of DNA at high relative humidities. It seems likely that local heterogeneity in dynamics and structure also occurs in base domains as well.

However, some lines of evidence indicate that the base domains are less mobile than the DNA backbone in the fiber;[3] in crystals,[4] and in solution.[5,6] Since ^{31}P NMR only provides information about the phosphodiester groups of DNA, a question arises as to whether the conclusions derived from ^{31}P NMR studies correctly represent the nature of the base domains as well. It is therefore important to examine the base domains of DNA using the same conditions as employed in ^{31}P NMR studies. ^{2}H NMR is the most suitable probe if nonexchangeable protons of the bases of DNA are selectively substituted by deuterium nuclei. DiVerdi and Opella[3] and later Bendel et al.[7] have reported the solid state ^{2}H NMR studies of DNA with the deuterium-labeled purine bases at C8 position. The former group has attributed the different behavior of ^{2}H and ^{31}P NMR line-shapes, observed when humidity or temperature was changed, to differences in flexibility in the backbone and base domains in hydrated DNA. We have pointed out, however, that differences in the ^{2}H and ^{31}P NMR time scales must be taken into account in the line-shape analysis, and we also suggested that more clear-cut conclusions could be obtained if oriented DNA samples were used for NMR measurements.[2] Recently Vold et al.[8] reported a ^{2}H NMR study of oriented Na- and Li-DNA samples at relatively low humidities.

In the present study, we prepared highly oriented Na-DNA fibers by the traditional method, rather than the wet-spinning technique[9] employed by Vold et al.[8] that produces a block of oriented sample. Humidity control is easier for the fibers than for the DNA block because of the size of sample. We report measurements of ^{2}H NMR spectra and relaxation times of two typical forms of DNA, i.e., the A and B forms of oriented DNA fibers with deuterated purine bases, and discuss the structure and dynamics of DNA fibers. We also show that ^{2}H and ^{31}P NMR results are consistent and discuss the relative merits of ^{2}H and ^{31}P NMR spectroscopy for studying DNA.

Experimental

Deuterium Labeled Nucleosides and DNA. Lyophilized salmon sperm DNA was purchased from P. L. Biochemicals and purine nucleosides were from Sigma Chemical company U.S.A. Nucleosides were dissolved in D_2O, heated at 90 °C for 6 h, dried in vacuo. They were redissolved in H_2O and dried, and this procedure was repeated several times to remove the residual D_2O. The degree of deuteration at C8 position was checked by ^{1}H NMR. Deuterium exchange of C8 position of purine bases of DNA was carried out following the method of tritium exchange of DNA by Doppler-Bernardi and Felsenfeld.[10] Lyophilized DNA was dissolved overnight into D_2O (10 mM††† Tris-HCl, 1 mM EDTA, pH 7.5) and then the solution was mixed with 4.0 M NaCl and 10 mM Tris in D_2O to a final concentration of 2.0 M NaCl and 10 mM Tris (pH 7.5). The solution was held at 83 °C in a thermostat for 10 h and then cooled in an ice bath. Two volumes of cold ethanol were added, yielding a fibrous precipitate. The precipitate was dried in the atmosphere and redissolved in H_2O, 10 mM Tris, at 4 °C overnight. The solution was exaustively dialyzed against H_2O, 10 mM NaCl, for 2 d.

††† 1 M=1 mol dm^{-3}

DNA Films and Fibers. The solution of DNA (1—2% w/v) prepared above was centrifuged at 50000 rpm for 16 h, yielding viscous pellets. The viscous pellets were dried on a plastic sheet in the atmosphere to make films, and many pieces of films were collected for NMR measurements. To make an oriented fiber a drop of the pellet was placed between the ends of tooth picks and left to dry in the atmosphere. Optically homogeneous fibers of 3—5 mm length and 0.1—0.3 mm diameter were usually obtained. The fibers gave excellent X-ray fiber diffraction images of the A type of DNA at 79% RH.

NMR Measurements. About 40 fibers were aligned in parallel on a sample holder and held overnight for equilibration in the atmosphere of a given humidity. The DNA films prepared above were also equilibrated at an appropriate humidity before NMR measurements. Relative humidity was controlled by saturated solutions of various salts.[11] The sample holder was inserted into a sample tube (6 mm o.d.), capped tightly and mounted on a goniometer. ^{2}H NMR spectra were obtained at 38.45 MHz with a quadrupole echo pulse sequence, 90°-τ-90°-τ-FID, on a home-built spectrometer with a NIC-1280 computer (for details, see Sarkar et al.[12]). Some ^{2}H NMR spectra were observed at 76.77 MHz on a NIC-500 spectrometer (Nicolet Inc.). The 90° pulse was 2.5 μs at 38.45 MHz and 3.0 μs at 76.77 MHz. A time interval of 50 μs was used between the two pulses of the echo sequence, unless otherwise stated, to eliminate the base line distortion caused by probe ringing after the pulse.

Relaxation times T_1 and T_2 were measured by a progressive saturation method with variable repetition time, and a quadrupole echo with variable pulse interval, respectively. All measurements were performed at 22 °C.

Analysis of ^{2}H NMR Spectra

In the case of the ^{2}H nucleus the solid-state NMR spectrum is dominated by the electric quadrupole interaction whose coupling constant for ^{2}H in C-^{2}H bonds e^2qQ/h=180 kHz can lead to spectra up to 250 kHz wide. As a result, the quadrupole echo spectrum may be distorted depending on the ^{2}H relaxation time and the instrumental performance. A general rewiew of ^{2}H NMR spectroscopy[13] has appeared recently and spectral distortions have been analyzed in detail, taking into account the effect of relaxation (Spiess and Sillescu[14]) and finite pulse power (Bloom et al.[15]).

Analysis of the ^{2}H NMR spectrum from an axially oriented specimen is straightforward. By the analogy with the analysis of the ^{31}P NMR from oriented DNA fibers,[1,16] the frequencies of the observed quadrupolar split lines, $\nu^{obsd}(\phi,\alpha)$, are give by

$$\nu^{obsd}(\phi,\alpha) = \pm\sqrt{\frac{3}{2}}\frac{eQ}{2\hbar}\rho_{20}^{lab}$$

$$\rho_{20}^{lab}(\phi,\alpha) = \sum_{m,n,p=-2}^{2} D_{p0}^{(2)}(0,\phi,0)D_{np}^{(2)}(\phi,\theta,0)D_{nm}^{(2)}(\alpha,\beta,\gamma)\rho_{2n} \quad (1)$$

for ^{2}H with I=1. Here $D_{nm}^{(2)}(\alpha,\beta,\gamma)$ is the Wigner rotation matrix of the second rank, ρ_{2n} is the irreducible EFG tensor in the principal axis system[17] and the Euler angle notations are given in Figs. 1A and B. The principal axis system of the tensor is defined by Euler angles α, β, and γ with respect to the molecular fixed axis system (a, b, c). The fiber axis system is taken to be identical to the goniometer axis system (Fig. 1B). The goniometer angle ϕ between the fiber axis and the magnetic field direction can be varied. The spectrum in which ϕ=0° corresponds to the spectrum of the fibers oriented parallel to the magnetic field, and the spectrum at ϕ=90° is that of fibers oriented perpendicular to the field. The angle θ is the angle made by the molecular fixed axis, c, and the fiber axis. As described previously,[1] the spectrum at a given ϕ can be calculated by the superposition of the spectral lines with appropriate weights over all the possible angles for α, β, γ, θ, and ψ. Gaussian distributions were also assumed for β and θ with standard deviations, $\langle\Delta\beta\rangle$ and $\langle\Delta\theta\rangle$, respectively.

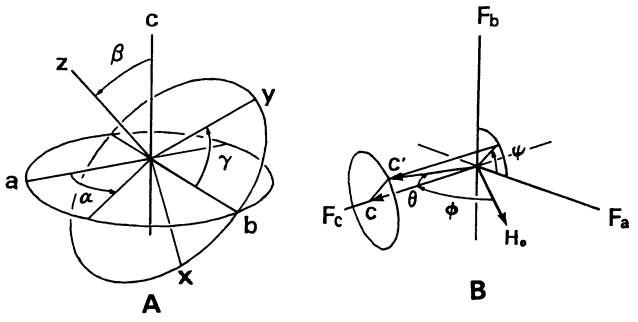

Fig. 1. Coordinate systems. (A) System (a, b, c) represents the molecular fixed system and axis c coincides with the helix axis of DNA. The principal axis system (x, y, z) of the quadrupolar coupling tensor is described by Euler angles α, β, and γ with respect to the molecular axis system as a reference frame. (B) Vector c lies along the fiber axis and vector c′ represents the helix axis of misaligned DNA which makes an angle θ with respect to the fiber axis. H_0 is the magnetlc fieid and ϕ is the goniometer angle.

Results

The degree of deuteration at the C8 positions of guanosine and adenosine was estimated to be about 70 and 75%, respectively, from the signal intensity in the ^{1}H NMR spectrum of each molecule in $CDCl_3$. According to the tritium-exchange reaction[10] of DNA in D_2O solution at 83 °C for 10 h, 30% of the total number of guanosine and adenosine residues, about 20% of the adenosine and 40% of the guanosine residues, were assumed to be deuterated. No appreciable degradation was expected under our experimental conditions.

^{2}H Powder Pattern Spectra. The spectra from polycrystalline guanosine and adenosine exhibited a typical static powder pattern with a quadrupolar splitting of 126±2 kHz as shown in Fig. 2. The long relaxation time T_1 of these samples at 22 °C, 30±10 s, indicated that the molecules were virtually immobile. It is interesting to note that the sugar ring of deoxyguanosine is found to be mobile.[18] The best visual fit with the observed spectrum of adenosine, shown in the bottom of Fig. 2, yielded values of principal frequencies of the quadrupolar coupling tensor, ν_1, ν_2, and ν_3, of −72±1, −63±1, and 135±3 kHz, respectively. The resulting nuclear quadrupole coupling constant and asymmetry parameter were 180±3 kHz and 0.06—0.07(±0.01), respectively. On the other hand, the parameter η of guanosine was a little larger than that of adenosine but it was in the above error range. The non-zero asymmetry parameter observed for the C8-^{2}H bonds in guanosine and adenosine is not surprising because a value of η=0.07—0.08 have been reported for the C2-^{2}H bonds in imidazole and benzimidazole at 77 K by Hiyama et al.[19]

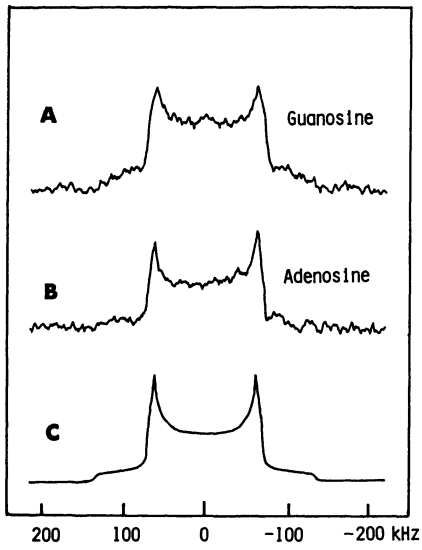

Fig. 2. Quadrupole echo ^{2}H NMR spectra (38.5 MHz) of polycrystalline guanosine (A), adenosine (B), and simulated powder pattern (C) at 22 °C. Measurement conditions: Spectral window 1 MHz; data points 4096 with zero filling; pulse width 2.2 μs; pulse interval 50 μs; delay time 40 s; exponential line broadening 2 kHz; scan number 1600. The simulated spectrum (C) was calculated using line broadening of 2 kHz and the principal values, 132, −64, and −70 kHz for ν_3, ν_2, and ν_1, respectively.

Figure 3 shows the ^{2}H powder patterns of DNA films, labeled at C8 position of guanosine and adenosine, at 79 and 92% RH. Note that a spike at the center of each spectrum arises from the natural abundance of deuterium water in the sample. At each relative humidity, two spectra were obtained by the quadrupole echo method with different pulse intervals (the time between $\pi/2$ pulses). The top spectrum was obtained with a 50 μs pulse interval and the bottom was with a 100 μs interval.

The reduction of signal intensity observed at 79% RH with the larger interval is a consequence of the finite T_2 value (200 μs) of the sample. The anisotropy in T_2 is evidently quite small, since the line-shapes observed using both pulse intervals are virtually identical to those of guanosine and adenosine (Fig. 2). The quadrupolar splitting (123±2 kHz) is slightly smaller than that observed for adenosine (126±1 kHz). The large splitting observed in the DNA sample shows that the amplitudes of molecular motions are small, and the small intensity losses (due to finite T_2) show that the amplitudes of motion on the time scale of ω_Q^{-1} (10^{-4}—10^{-6} s) are insignificant.

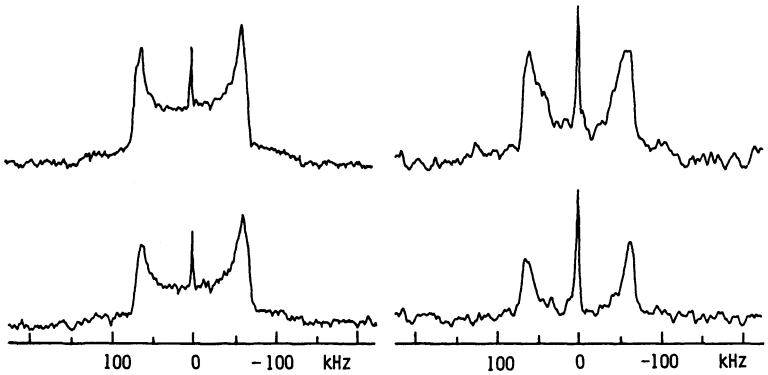

Fig. 3. Quadrupole echo spectra (38.5 MHz) of ^{2}H NMR of nonoriented DNA films at 79% RH (left) and 92% RH (right). Top and bottom spectra were taken with different pulse intervals, 50 and 100 μs, which may be used for examining the anisotropy effect of the relaxation time T_2. The measurement conditions were almost the same for all the samples as in Fig. 2, with delay time 0.6 s and scan number 32000.

Although the line-shape and T_2 data at 79% RH are similar to those observed in the model compounds, the measured T_1 is much smaller in DNA than in the model bases (Table 1). This implies that a small amplitude motion is taking place on the time scale of ω_0^{-1} (10^{-7}—10^{-10} s) in the 79% RH sample.

We can obtain an estimate of the amplitude of this motion by comparing the observed quadrupole splitting in DNA with the splitting observed in the model compounds. $2|\nu_1|$ and $2|\nu_2|$ (the quadrupole splittings) in DNA were smaller by 5.0 (±1) and 4.0 (±1) kHz, respectively, than in the model compounds. Because the reduction in splittings is much less than $2|\nu_1|-2|\nu_2|$ for either guanosine or adenosine, 16 kHz, there is little likelihood of the principal axis interchange between the nucleoside and DNA. Therefore, we make the reasonable assumption that the orientation of the nonaveraged ^{2}H EFG tensor is the same in the nucleoside and in the DNA molecule.

Table 1. Relaxation Times T_1 and T_2 of ^{2}H NMR at 38.5 MHz of Unoriented DNA Films and Oriented DNA Fibers at 22 °C

Relative Humidity/%	T_1/ms[a]		T_2/μs[a]	
	DNA Film	Fiber[b]	DNA Film	Fiber[b]
75	177±20	—	164±15	—
79	167±13 (480±80)[c]	158±25	183±20	—
87	75±9	—	134±14	—
92	30±6 (71±14)[c]	25±7	96±9	100±20
98	16±3	—	101±8	—

a) Relaxation times measured for the perpendicular component of the tensor in the powder pattern spectrum of DNA films. b) Relaxation times measured for the parallel spectrum of DNA fibers. c) T_1 values measured at 76.8 MHz.

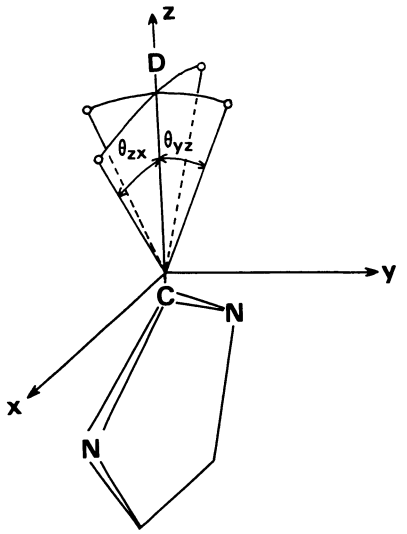

Fig. 4. Principal axis system (x, y, z) of the EFG tensor relative to the atomic coordinates of a purine base, and two sets of two jump sites for the C8-^{2}H bond whose fluctuation amplitude are represented by θ_{zx} and θ_{yz}.

The reduced splittings were analyzed using the four-site jump model illustrated in Fig. 4. If the jump rate is much larger than ω_Q, the motionally averaged principal frequencies, $\bar{\nu}_1$ and $\bar{\nu}_2$, are related to the motional amplitudes, θ_{zx} and θ_{yz}, by following equations

$$\bar{\nu}_1 = \frac{1}{2}(\nu_1+\nu_1\cos^2\theta_{zx}+\nu_3\sin^2\theta_{zx}), \quad (2)$$

$$\bar{\nu}_2 = \frac{1}{2}(\nu_2+\nu_2\cos^2\theta_{yz}+\nu_3\sin^2\theta_{yz}). \quad (3)$$

Using these equations together with two sets of the observed values of ν_i and $\bar{\nu}_i$ (i=1, 2), θ_{yz} and θ_{zx} were calculated to be 8.1° and 8.9°, respectively. Since these angles are nearly equal, the motion does not appear to have a preferential axis.

As mentioned earlier, the spin-lattice relaxation time, T_1 at 79% RH, 167 ms, is substantially shorter than that found in the nucleosides. The possible minimum T_1 resulting from dipole-dipole interactions with the nearest neighbor protons is about 10 s. Therefore, T_1 is determined by quadrupolar relaxation.

Since in the line-shape we assumed that the rotation about a single axis is not likely at 79% RH, we analyse the measured T_1 values at 38.5 and 76.8 MHz using the diffusion-in-a-cone model. In this model we assume that the C-^{2}H bond diffuses uniformly over the surface of a cone having semiangle θ_0. In this case the rms fluctuation in θ is $\sqrt{\theta_0^2/2}$, and θ_{rms} is taken to be the average of θ_{zx} and θ_{yz}, 8.5°, calculated from the line-shape analysis. Using the general formalism of Torchia and Szabo[17] together with the condition that $\theta_{rms}^2 \ll 1$, we find that

$$\left\langle \frac{1}{T_1} \right\rangle = (\omega_Q^2/10)\sin^2 2\theta_{rms}[g(\tau, \omega_0) + 4g(\tau, 2\omega_0)], \quad (4)$$

where $\langle\cdots\rangle$ indicates the average overall orientations of the field relative to a crystal fixed axis system, and $g(\tau,\omega)$ equals $\tau(1+\omega^2\tau^2)^{-1}$. In writing Eq. 4 we have made the approximation that η equals zero and $\omega_Q/2\pi$ is taken to be the average of $2|\nu_1|$ and $2|\nu_2|$ of the nucleoside. Inserting the measured values of $\langle 1/T_1\rangle$, ω_Q, θ_{rms}, and $\omega_0/2\pi$ (38.5 MHz) into Eq. 4 we obtain two solutions τ_c=2.5×10^{-10} and 3.6×10^{-8} s. Only the larger value is consistent with the observation of a strong frequency dependence of T_1 (Table 1). The ratio of T_1 at 76.8 MHz to T_1 at 38.5 MHz was 2.9±0.5 which was a little smaller than the theoretical value of 4.0.

In contrast with the 79% RH DNA spectra (left, Fig. 3) the 92% RH DNA powder patterns (right, Fig. 3) are distinctly different from the static powder patterns of the model compounds, Fig. 2. Only the perpendicular components of the pattern have significant intensity. This observation suggests that the DNA molecule reorients about the helix axis with a correlation time in the 10^{-4}—10^{-7} s range. Such a motion would cause T_2 to be very short for all orientations of the field relative to the helix axis, except for the field parallel to the rotation (helix) axis.

In order to estimate the rate and amplitude of the axial motion, the observed spectra were simulated using an N-site jump model, in which jumps were restricted to nearest neighbors (Fig. 5). The computer program includes correction for the relaxation effect on the line-shape of quadrupole echo spectrum as reported by Speiss and Sillescu.[14] Six sites are sufficient to approximate rotational diffusion, and limit computational costs. Figure 6 shows spectral shape as a function of maximum rotation amplitude (i.e., 5 ω). Each jump rate, k, shown in the figure legend was estimated so as to give a reasonable fit to the observed spectra (right, Fig. 3). It was found that a maximum rotation amplitude of 150°, i.e., root mean square fluctuation for each jump Φ_{rms}, of at least 30°, was required to simulate the observed 92% RH DNA spectrum. As expected, the jump rate required to fit the spectrum, $(2\pm1)\times10^6$ s^{-1}, was approximately equal to $\omega_Q/2\pi$. Motions on the time scale of ω_Q result in anisotropic T_2 values which cause intensity losses and line-shape distortions in ^{2}H spectra. Although satisfactory spectral simulations were also obtained with values of Φ_{rms} greater than 30°, again with the jump rate approximately equal to ω_Q, these solutions were ruled out by the ^{31}P spectra of 92% RH DNA. These spectra showed that the ^{31}P chemical shift anisotropy, $\sigma_{33}-\sigma_{11}$, observed in the spectra of 92% RH at 24 MHz (Shindo et al.[20]) and 40 MHz (not shown) were only 12% smaller than that in the spectra of the 79% RH DNA. Futhermore, by applying a restricted rotation model with a rectangular potential well to the ^{31}P NMR data of DNA fibers at 92% RH, the average rotation amplitude about the helix axis was estimated to be about 130° (Fujiwara and Shindo[2]). This amplitude is close to the maximum rotation angle of 150° as estimated above. Therefore only the motions of restricted six-site jump model having a root mean square fluctuation less than 35° are consistent with the ^{31}P data.

Although the restricted, slow azimuthal reorientation of the DNA molecule accounts for the line-shape observed at 92% RH, this motion does not explain

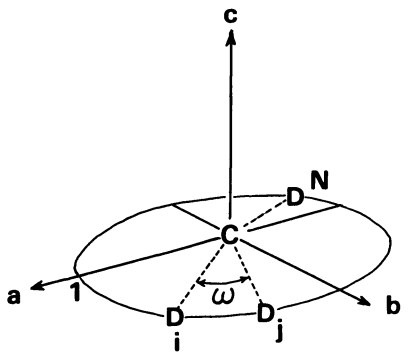

Fig. 5. N site jump model for rotational motion about the helix axis. Jumping is allowed only between nearest neighbors with jump rate k and jumping matrix $R_{i,i+1}=k$. For free jump, site N+1 equals site 1, allowing $R_{1N}=R_{N1}=k$. For restricted jump, $R_{1N}=R_{N1}=0$.

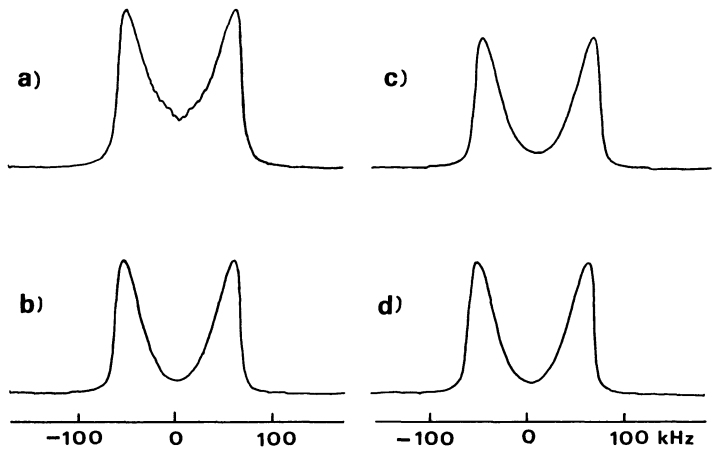

Fig. 6. Calculated ^{2}H NMR powder patterns based on the six-site jump model, (a) $5\omega=100°$, $k=2.5\times10^6$ rad s^{-1}, (b) $5\omega=150°$, $k=3\times10^6$ rad s^{-1}, (c) $5\omega=200°$, 1.5×10^6 rad s^{-1}, (d) $5\omega=250°$, $k=10^6$ rad s^{-1}. Angle ω represents the azimuthal angle between nearest neighbor sites as in Fig. 5.

either the small average T_1 value, Table 1, measured at 92% RH or the 5 kHz reduction in $2|\nu_1|$ observed when RH is increased from 79 to 92%. The rotational motion is too slow, i.e., the correlation time is too far from the T_1 minimum, to account for the small T_1 value, and the motion about the helix axis does not affect the value of $2|\nu_1|$ regardless of time scale. The reduction of $2|\nu_1|$ with increasing RH can be explained by assuming that the amplitude of the C-^{2}H bond fluctuation in the cone, θ_{rms}, increases from 8.5 to 13.5° upon increasing RH from 79 to 92%. Using this value in Eq. 5 together with the measured values of $\langle 1/T_1 \rangle$, ω_0, and ω_Q, we calculate $\tau_c = 1.5 \times 10^{-8}$ s, ca. one-half as large as found at 79% RH.

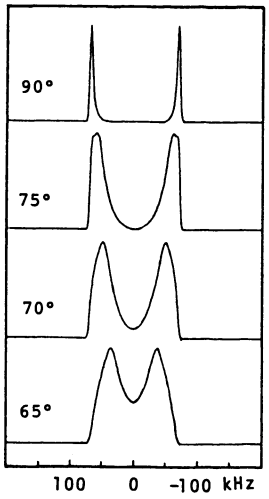

Fig. 7. Effect of Euler angle, β, and the distribution of $\langle \Delta\beta \rangle$ and $\langle \Delta\theta \rangle$ on the spectrum of DNA fibers oriented parallel to the magnetic field direction. A series of spectra show the dependence of spectral pattern on Euler angle β with fixed parameters, $\langle \Delta\beta \rangle = 3°$, $\gamma = 0°$, $\langle \Delta\theta \rangle = 8°$

^{2}H Spectra of Oriented DNA Fibers. Before presenting the experimental spectra of axially aligned DNA fibers we first present theoretical spectra which demonstrate how Euler angle β and standard deviations of β and θ affect the line-shape. The standard deviations $\langle \Delta\theta \rangle$ and $\langle \Delta\beta \rangle$ represent the imperfect alignment of molecules along the fiber axis and heterogeneity of the local structure, respectively. In calculating the theoretical spectra, the orientation of the ^{2}H EFG tensor was assumed to be as shown in Fig. 4, and effects of molecular motion on the line-shape were included with use of motionally averaged principal frequencies.

Figure 7 shows a series of the theoretical spectra calculated for DNA fibers oriented parallel to the magnetic field. The spectra in Fig. 7 demonstrate how the line-shape changes as a function of Euler angle β. The values of the other parameters were goniometer angle $\phi = 0°$, standard deviations $\langle \Delta\beta \rangle = 3°$ and $\langle \Delta\theta \rangle = 8°$, $\gamma = 0°$ and line-width=2 kHz. As expected, the dependence of the peak separation on β is weak around ca. 90°, and becomes stronger as β decreases. It is important to note that at $\phi = 90°$ the spectrum (top, Fig. 7) is hardly affected by either $\langle \Delta\beta \rangle$ or $\langle \Delta\theta \rangle$.

We now turn to an analysis of the experimental spectra. Figure 8 shows the observed and simulated spectra for the fibers at 79% RH oriented parallel, at 45°, and perpendicular to the magnetic field. The fibers at this humidity gave a typical A form of DNA as judged from the X-ray fiber diffraction patterns. Simulated spectra, which reproduced the observed spectra, were calculated using the parameters, $\beta = 70°$, $\langle \Delta\beta \rangle = 3°$, $\gamma = 0°$, and $\langle \Delta\theta \rangle = 8°$. Simulated spectra include 10% of the B form in the A form of DNA,

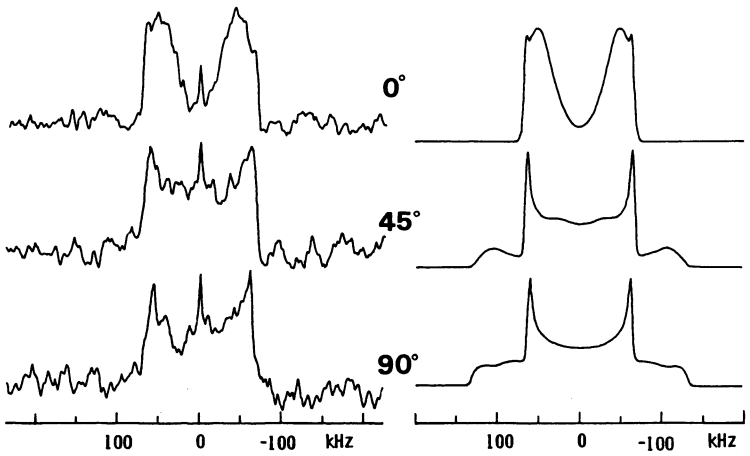

Fig. 8. Quadrupole echo spectra (left) of ^{2}H NMR (38.5 MHz) of oriented DNA fibers at 79% RH at different orientations of fiber axis to the magnetic field as indicated, and simulated spectra (right). Measurement conditions are the same as in Fig. 2, except for pulse width 2.5 μs, delay time 0.3 s, line broadening 2 kHz, scan number 120000—160000.

which gives small peaks at the edges of both sides in the parallel spectrum. The signal components beyond ca. 100 kHz from the carrier were attenuated, because of the finite pulse power of the instrument ($\pi/2$ pulse with 2.5 μs) and were not detectable above the noise level. The standard deviation in θ, $\langle\Delta\theta\rangle=8°$, was estimated from the ^{31}P NMR of the same sample (data not shown), and was close to the value of 10° reported previously (Shindo et al.[1]). The value obtained for β, 70°, has an experimental error of less than three degrees, because the peak separation is very sensitive to the value of β, where β is in the range 65°—75°, Fig. 7. The narrow distribution of β is consistent with the fact that the A form of DNA has a very narrow conformational distribution,[1,16] i.e., the structure is independent of the base composition and sequence.

Although we were able to simulate the 79% RH fiber spectra using the static quadrupole coupling obtained for the model compounds, the splittings in the calculated fiber spectra were always larger than the measured splitting. The best simulations of those measured DNA fiber spectra at 79% RH were obtained using a quadrupole splitting that was 4 kHz smaller than that measured in the model compounds. This result confirms the conclusion, obtained from the analysis of the spectra of DNA films, that a small amplitude motion $\theta_{rms}=8.5°$ takes place in DNA at 79% RH. Further support for this conclusion is provided by the small T_1 value observed in the fiber sample, Table 1, which agreed (within experimental error) with the measurement of T_1 at the perpendicular component in the spectrum of DNA films at 79% RH. Finally we note that because the small amplitude motion takes place on a rapid time scale, $\omega_Q\tau_c\ll1$, T_2 is large for all orientations relative to the external magnetic field H_0. For this reason, the same relative intensity was observed in the spectra of all three orientations shown in Fig. 8.

Figure 9 shows the observed and simulated spectra of the fibers at 92% RH oriented parallel, at 45°, and perpendicular to the magnetic field. All the spectra were measured under the same conditions and were recorded in the absolute intensity mode. The simulated spectra are normalized to have a constant spectral area. The fibers gave an X-ray pattern of the B form of DNA, and therefore we used the X-ray structure of the B form of DNA (Arnott et al.[21]), together with the results of ^{31}P NMR (Shindo et al.[1]) to set initial parameters for simulating the spectra in Fig. 9: The base plane was nearly normal to the helix axis, i.e., $\beta=90°$ and $\gamma=0°$ with standard deviations $\langle\Delta\beta\rangle=10°$ and $\langle\Delta\theta\rangle=8°$. The peak separation measured for the parallel spectrum was 132±2 kHz, corresponding to the peaks at ±66 kHz. The value of 66 kHz is closer to the principal value $|\nu_1|$ than $|\nu_2|$. Considering that the base plane is nearly perpendicular to the helix axis, the axis of ν_1 must be approximately parallel to the helical axis, i.e., $\beta=90°$ and $\gamma=0°$ or 180°. In agreement with the results obtained at 79% RH the best simulation of the observed splittings at 92% RH was obtained by assuming principal values of the EFG tensor were reduced from their static values by small amplitude motion, $\theta_{rms}=13.5°$. The T_1 value observed for the oriented fibers (parallel orientation) is listed in Table 1 where it is seen that the T_1 values measued for the fibers and films at 92% RH are in agreement within experimental error. This result confirms that a small amplitude motion occurs in the fibers. The parallel spectrum is quite sharp (natural line-width ca. 6 kHz)

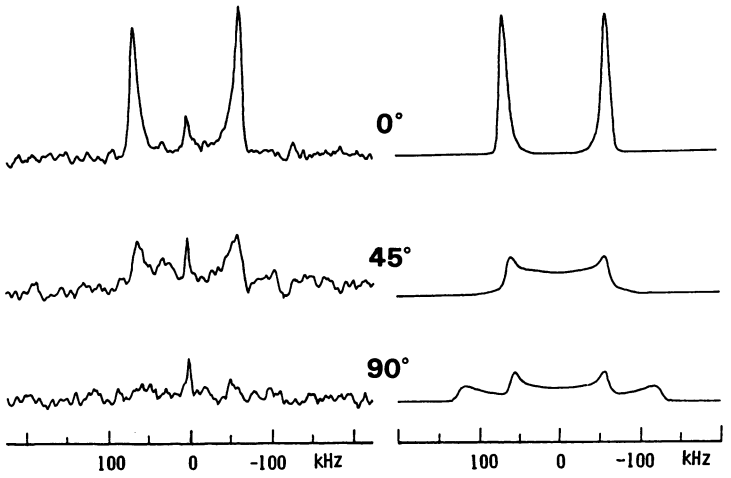

Fig. 9. Quadrupole echo spectra (left) of the ^2H NMR of oriented DNA fibers at 92% RH and at different orientations of the fiber axis, and simulated spectra (right). All the spectra were obtained under the same conditions as in Fig. 6, with delay time 0.3 s and scan number 112000.

in spite of the presence of fairly wide distributions of β and θ. This observation is caused for the fact that the peak positions are very insensitive to changes in β and θ when β=90°, as mentioned above (Fig. 7). In contrast with the results obtained at 79% RH, large intensity losses were observed when the sample was oriented at 45 and 90° with respect to the field, Fig. 9. In fact, at 90° orientation, the signal intensity was not above the noise level, and at 45°, the signal was observed in the neighborhood of the perpendicular components of the spectrum ($\nu \approx \pm 65$ kHz). These observations are explained by the slow reorientation of the DNA about the fiber axis (rms fluctuation 30°, $\tau_c \approx 10^{-6}$ s) discussed in conjunction with the analysis of the line-shape of the DNA films at 92% RH. This motion will make the ^{2}H T_2 value very short for all orientations of the fiber axis relative to the field, except for the parallel orientation only where the axis of motion (the fiber axis) is parallel to the field. Therefore spectral intensity at this orientation (where $\nu \approx \pm 65$ kHz) only remains unattenuated in the quadrupole echo experiment.

The spectra of oriented DNA fibers at 98% RH (not shown) were similar to those observed at 92% RH. However, the T_1 measured at 98% RH, Table 1, was about one-half as large as measured at 92% RH. Since the splitting observed in the parallel spectra at 92 and 98% RH was the same, the value of θ_{rms} was the same at these RH. Therefore the samller T_1 value observed at 98% RH was due to a shorter correlation time, i.e., $\tau_c = 6 \times 10^{-9}$ s.

Discussion

Powder Pattern Spectra of Purine Bases. According to recent communication from Vold and coworkers,[22] they observed two types of deuterons in polycrystalline guanosine deuterated at the C8 position and they attributed this to two different conformations of guanosine in the unit cell. As shown in Fig. 2, however, the powder pattern of guanosine is rather normal, although the peak is a little broad compared with that of adenosine. The difference between the spectra of Tsang et al.[22] and ours is probably accounted for by the different cystal forms of guanosine. For many other purine bases, including those of 5′-AMP and 5′-GMP, the static quadrupolar coupling constant, $e^2qQ/h = 179 \pm 2$ kHz and asymmetry factor η=0.06—0.07 (±0.01), appeared to be characteristic of the 8-position in all the purine derivatives. These parameters are in good agreement with those determined by us.

Structure of the Base Domain. Previously reported ^{31}P NMR studies of DNA fibers,[1,5,20,23] have shown that the backbone conformation of the B form of DNA is very heterogeneous, i.e., Euler angles β and γ (structural parameters of the phosphodiester) had a wide distribution of values with standard deviation 5—15° at 92% RH, whereas the backbone of the A form had nearly a single conformation. As for the base domain, according to the structural models inferred from X-ray fiber diffraction studies,[21] the base planes of the A form of DNA tilt by ca. 70° and roll by several degrees with respect to the axis normal to the helix axis, while the base planes of the B form of DNA are almost perpendicular to the helix axis. Assuming that the C8–H bond axis of the purine bases lies on the bisector of the angle made by the base atoms, N7–C8–N9, Euler angles β and γ were calculated using the atomic coordinates of the DNA structure. Because of the small asymmetry factor, η=0.06, the ^{2}H spectrum was only slightly affected by changes in γ. The angle made by the C8–H bond axis and the helix axis was calculated to be 67.8° (or 112.2°) and 96.1° (or 83.9°) for the A and B forms of DNA, respectively. The values in the parenthesis represent the angles for the antiparallel chain in the duplex. (Both chains give the same spectrum because of the diad symmetry of the DNA model.) On the other hand, the observed value was 70° (at most 73°) and 90°±10 for the A and B forms, respectively. The observed and calculated values agreed with each other within the experimental error. This was also the case for Fuller's B-DNA model[24] as seen in Table 2. Levitt's model[25] gave a value (73.2°) close to the averaged value (75.2°) for the 10 independent purine bases of the single crystal structure[26] of r(GCG)d(AATTCGC). The values of β calculated for two oligonucleotides in the A family of DNA are within the range from 70 to 80°, and their averaged values are slightly higher than the observed value. Several possible reasons for this discrepancy are as follows, 1) the principal axis z of the quadrupolar tensor is not exactly along the C8–^{2}H bond, 2) the C8–^{2}H bond does not lie on the bisector of the atoms, N7–C8–N9, and 3) the average structures of DNA in the fiber and oligonucleotides are not identical. As for the first item, although the orientation of the z

Table 2. Euler Angles β and γ Calculated from the Atomic Coordinates of DNA Models and the Single Crystal Structures of Oligonucleotides[a]

A Form DNA		B Form DNA		Ref./Sample
β	γ	β	γ	
67.8	4.9	96.2	0.1	21/Fiber
73.2±0.2	12.1	97±6	15.5±0.8	25/Fiber
—	—	90.4	4.8	34/Fiber
68.9	0.6	—	—	24/Fiber
75.9±5	5.2±4.3	—	—	35/Octamer
75.2±2.8	8.5±3.5	—	—	36/Decamer
108.1±2.6	174.9±4.4	—	—	
—	—	97±15	18±12	4, 32/
—	—	84±15	163±12	Dodecamer

a) See the data of oligomers in the text.

principal axis is not known for the purine ring, all of the single crystal ^{2}H NMR studies[27-30] have shown that the major principal axis of the ^{2}H bonds is along the C–H bond axis within a couple of degrees. As for the second item, the neutron diffraction study[31] of 1-methylthymine:9-methyladenine crystal has shown that the C8 proton is not on the bisector of the atoms, N7–C8–N9, although the purine base is planar. That is, the C8–H bond axis inclines by about 1.7° from the bisector towards atom N9. Using this result, the Euler angle β was recalculated for the decamer, r(GCG)d-(AATTCGC), and its averaged value changed by only 0.1°. With regard to item three, it is often difficult to determine the helix axis of small oligonucleotides such as those mentioned above. Furthermore, the helix axis often bends, probably due to molecular packing forces.[4] We believe that this factor is the major reason for the discrepancy between the value of β calculated for oligonucleotides and that observed for the fibers.

It is interesting to note the conformational variation in the base planes in the single crystals of oligonucleotides, such as a decamer in the A family of crystalline form of DNA,[26] and two dodecamers, d(CGCGAATTCGCG) and d(CGCGAATTBr5CGCG) in the B family of the crystalline form of DNA.[32] The β values of the decamer are in a very narrow range of a few degrees (±3°), whereas those of the dodecamers are significantly varied over the range of ±15° from the average value. These results indicate that the bases of the A form of DNA are restricted to nearly a single conformation, while those of the B form of DNA have a wide distribution of conformations. The results derived from line-shape analysis of the quadrupole spectra of the A and B DNA fibers are consistent with the above mentioned.

In conclusion, the ^{2}H NMR spectra of DNA fibers studied here demonstrated that the orientations of the bases in the A form of DNA were constant within a few degrees in Euler angle β, whereas value of β in the B form of DNA varied over a range of ±10°. These results are consistent with the distribution of the Euler angles β and γ, calculated on the basis of the structure of oligonucleotides (see Table 2), and they are also consistent with structural features of DNA deduced from ^{31}P NMR studies. It should be noted that the structural information that can be obtained from the spectra of the C8–^{2}H label is limited. Accurate values of γ are not attainable because η is very small, and the values of β and $\langle\Delta\beta\rangle$ have large uncertainties (±7°) in the case of B-DNA because β is ca. 90°, a value in the range where the line-shape and peak splitting are least sensitive to changes in β. More comprehensive and accurate information would be obtained by using synthetic DNA molecules ^{2}H labeled at another position.[18]

Dynamics of the Base Domain. As described earlier in this paper, the relatively small ^{2}H T_1 values observed at 75—79% RH, Table 1, are a consequence of rapid ($\tau_c=3.8\times10^{-8}$ s) motions of the C8–^{2}H bond axis. The line-shape and T_1 data are both well fit using a model in which the orientation of C8–^{2}H bond axis fluctuates in a cone (normal to the helix axis) throughout a rms angle of 8.5°. The reductions in T_1 value and peak splitting observed at higher relative humidity (92% RH) can be accounted for by a large fluctuation of the C8–^{2}H bond axis, rms angle of 13.5°, and smaller correlation time, $\tau_c=1.5\times10^{-8}$ s. It is interesting to note that the amplitude of the base fluctuation, 13.5°, estimated here for Na-DNA at 92 and 98% RH is almost identical to the value 10—13° estimated for Li-DNA at 66% RH by Vold et al.[8] These results suggest that the fluctuation amplitude of the base in the B form of DNA has only a weak humidity dependence over the range from 66 to 98% RH, although its correlation time has an appreciable dependence. The rapid, small amplitude fluctuation of the C8–^{2}H bond axis explains the humidity dependence of quadrupole splittings and T_1, but it does not fully explain the frequency dependence of T_2. Brandes et al.[33] therefore, suggested that a distribution of correlation times must be taken into account for this frequency dependence. We demonstrated that the large distortions and intensity losses in the quadrupole echo spectra of the oriented and unoriented DNA at 92% RH are explained by a slower ($\tau\approx10^{-6}$ s), larger root mean square amplitude (ca. 30°), reorientational motion of the C8–^{2}H bond axis about the helix axis. The six-site model employed here leads to a conclusion consistent with the rotational diffusion about the helix axis as discussed in the ^{31}P NMR study of DNA fibers.[1]

In the course of the present study, we learned that Dr. R. R. Vold in UC San Diego was studying oriented DNA samples by solid-state ^{2}H NMR. We greatly appreciate her kindness for sending us preprints in advance of publication. We thank Robert G. Griffin of MIT for the computer program to calculate spectra of the six-site jump model, and also thank Dr. L. Lerner of NIH for her reading of this manuscript and valuable discussions.

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