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Deuterium Relaxation and Internal Motion in Solid Li-DNA†

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ABSTRACT: As part of an effort to explore the nature of the internal motion in solid polynucleotides, the spectral densities of motion $J_1(\omega_0)$ and $J_2(2\omega_0)$ have been measured for oriented, partially hydrated samples of calf thymus Li-DNA deuterated in the guanine and adenine 8-positions. Both spectral densities increase with increasing hydration level, J_1 is found to be 2–5 times larger than J_2, and their frequency dependence appears to be ω^{-1} and $\omega^{-3/2}$, respectively. The large values of the ratio J_1/J_2 rule out any in-plane torsional motion as the dominant relaxation mechanism in these samples, but a drop in this ratio at high hydration levels (G13 H_2O/nucleotide) may indicate increasing contributions from such torsional motion. Although a satisfactory fit to a particular motional model has yet to be achieved, our findings show that the librational motion of the C_8–D bond at or below a hydration level of 10 H_2O/nucleotide is approximately uniaxial, with correlation times for the motion in the range 0.2–3.0 μs.

Dry DNA is very hygroscopic. It readily absorbs water from the atmosphere unit, and at 94–98% relative humidity, originally lyophilized DNA has the appearance of a viscous liquid. The absorption of water is accompanied by internal and/or overall motion of larger and larger amplitude, until in solution the DNA is free to reorient completely.

While numerous techniques exist for studying the dynamics of DNA in solution, magnetic resonance techniques are uniquely suited for determination of molecular motion in the solid state. Several NMR studies have been performed on solid polynucleotides (Shindo et al., 1980, 1985, 1987; DiVerdi & Opella, 1981; Nall et al., 1981; Opella et al., 1981; Bendel et al., 1983; Mai et al., 1983; Fujiwara & Shindo, 1985; Brandes et al., 1986, 1988a,b; Vold et al., 1986) and, recently, on solid oligonucleotides (Kintanar et al., 1989) with the objective of characterizing the modes of motion responsible for NMR line shapes and spin–lattice relaxation behavior. It is by now well documented that NMR spin–lattic relaxation times drop precipitously as water is adsorbed and that this more efficient relaxation is caused by librational motion of increasing amplitude. At high humidity slower, large-angle motion takes place that has profound effects on the NMR spectra. This information has chiefly been obtained by analysis of phosphorus and deuterium line shapes and relaxation rates. Phosphorus NMR has given information about backbone motion while the deuterium work, relying on deuterium labeling of the purines, has reported on the mobility of the bases. Previous deuterium NMR studies of oriented samples of Li-DNA (Vold et al., 1986; Brandes et al., 1988b) have demonstrated that the purine C_8–D bonds are oriented approximately perpendicular to the helix axis and that a hydration levels of less than ca. 13 molecules of water per nucleotide the motion is nearly uniaxial with an amplitude of less than 15°. At higher hydration levels large-angle motion seems to occur predominantly in the plane of the base pairs (Shindo et al., 1987). This motion may ultimately lead to the damped torsional deformation modes generally assumed (Allison & Schurr, 1979; Barkley & Zimm, 1979; Allison et al., 1982; Schurr et al., 1989) to be responsible for strongly nonexponential fluorescence depolarization in solution.

In an earlier study (Brandes et al., 1986) we measured the spin–lattice relaxation times of hydrated powders of Na- and Li-DNA at 38.4 and 76.8 MHz and attempted an interpretation of the results in terms of a simple diffusion in a cone model. The frequency dependence of the observed relaxation rates (ω^{-1}) could not be reconciled with the cone model, which predicts the relaxation rate to be proportional to ω^{-2} in the range of rates of interest. It was concluded (Brandes et al., 1986) that the weaker frequency dependence might be associated with the predominance of collective modes of motion of unspecified origin, and the present study was undertaken in the hope of improving our understanding of such processes. Thus, we have measured the individual spectral densities of motion which characterize the spin–lattice relaxation behavior of DNA deuterons in samples of oriented calf thymus DNA. The new data rule out damped torsional oscillations (Allison et al., 1982) of the bases about the base normal as the principal cause of relaxation at hydration levels below ca. 13 H_2O/nucleotide. We have consequently analyzed the spectral density data in terms of a simple diffusion in a cone model (Wang & Pecora, 1980; Lipari & Szabo, 1981), a more general cone model that allows for two different rates of motion, and a model that combines diffusive wobble of the base normal with torsional deformation. None of these models can fully account for the observed relaxation behavior, but simple

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Table I: Experimental Spin-Relaxation Times[a] Measured at 298 K and Two Spectrometer Frequencies for Solid Li-DNA Oriented with the Helix Axis Parallel to the Magnetic Field

relative humidity (%)	water content (H_2O/nucl)[b]	38.4 MHz			76.8 MHz		
		powder[c]	oriented sample		powder[c]	oriented sample	
		T_{1Z} (s)	T_{1Z} (s)	T_{1Q} (s)	T_{1Z} (s)	T_{1Z} (s)	T_{1Q} (s)
35	4.1	0.87 ± 0.04	0.90 ± 0.08	0.58 ± 0.05	2.0 ± 0.2		
66	7.7	0.20 ± 0.01	0.18 ± 0.01	0.11 ± 0.01	0.38 ± 0.02	0.43 ± 0.02	0.24 ± 0.01
75	9.9	0.15 ± 0.01	0.11 ± 0.01	0.080 ± 0.004	0.26 ± 0.02	0.26 ± 0.01	0.15 ± 0.01
84	13.4	0.09 ± 0.02[d]	0.042 ± 0.004	0.043 ± 0.003	0.20 ± 0.01		

[a] Experimental uncertainties listed are standard deviations from nonlinear least-squares fits to recovery and decay curves. [b] The uncertainty in the measured water content for these particular samples is estimated to be ≤±0.3 H_2O/nucleotide. [c] Data from Brandes et al. (1986) where the spin-lattice relaxation times were measured at the perpendicular edges of the powder patterns. [d] The water content of this powder sample was determined to be slightly lower, 12.1 ± 0.3 H_2O/nucleotide.

diffusion in a cone is as good as either of the two more complex models.

Materials and Methods

Oriented samples of Li-DNA, deuterated in the purine 8-position, were prepared as described elsewhere (Brandes et al., 1988a). The relative humidity (RH) used and the measured water content of the four samples are listed in Table I. Dueterium NMR spectra and relaxation times were recorded at 38.4 MHz on a homebuilt spectrometer and at 76.8 MHz on a GE 500 spectrometer equipped with a homemade deuterium probe, an ENI LPI-10 1-kW rf amplifier, and a Nicolet 2090 digital oscilloscope. The spectral densities of motion were determined from the recovery of Zeeman magnetization and the decay of quadrupolar order in phase-cycled (Vold, R. L., et al., 1981) Jeener–Broekaert experiments (Jeener & Broekaert, 1967).

Results and Discussion

Experimental Observations. In B-form DNA the purine C_8–D bonds are oriented at approximately 90° with respect to the helix axis. Therefore, the only orientation of DNA which results in a simple two-line spectrum is the one with the helix axis aligned parallel to the magnetic field. All other orientations lead to cylindrical powder patterns, with subsequent effective loss of a signal to noise in the NMR spectrum. Parallel alignment was consequently the only orientation for which the determination of individual spectral densities of motion was practical with the instrumentation currently availble. An example of the deuterium NMR spectra obtained during a Jeener–Broekaert experiment, $(\pi/2)_x$–τ_1–$(\pi/4)_y$–τ_2–$(\pi/4)_x$–acq, of oriented Li-DNA hydrated at 66% RH is shown in Figure 1. When the helix axis is parallel to the magnetic field, the two deuteron transitions are observed at $\nu_\perp = \mp(3/8)(e^2qQ/h)(1 - \eta_{eff})$, where $e^2qQ/h = 181 \pm 1$ kHz is the quadrupole coupling constant determined from mononucleosides (Tsang et al., 1987) and dry, "rigid" DNA (Brandes et al., 1986) and η_{eff} is an effective asymmetry parameter which includes the "rigid" value, $\eta = 0.07$, and the effects of librational motion on the electric field gradient tensor (Vold et al., 1986). The doublet splittings were observed to agree with those reported earlier (Brandes et al., 1988b) for this sample at this humidity. The earlier analysis showed that, at this degree of hydration, librational motion with an amplitude of 12 ± 2° takes place in a plane perpendicular to the helix axis, while the amplitude of the orthogonal libration is 10 ± 2°. The line shapes were analyzed as well (Brandes et al., 1988b) to yield a measure of the width of the helix axis alignment distribution combined with a distribution of the base tilt angles. The resulting distribution of the angle between the base normal and the magnetic field was found to have a mean squared deviation of 9°.

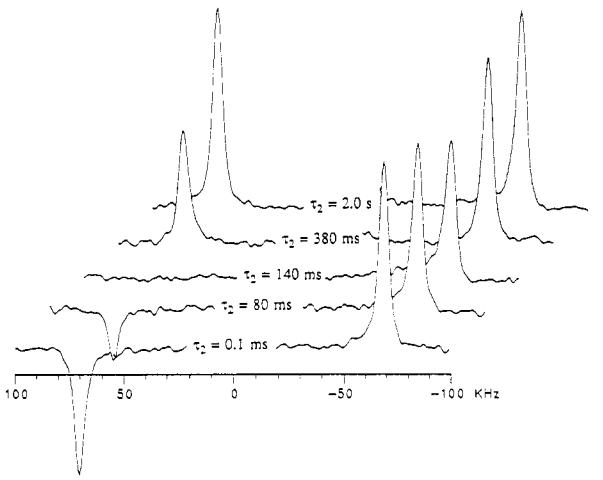

FIGURE 1: Deuterium NMR spectra obtained at 76.8 MHz of Li-DNA (50 mg) labeled in the purine 8-position and containing 7.7 H_2O/nucleotide (66% RH). The spectra were obtained in 8000 scans with the Jeener–Broekaert pulse sequence as a function of the pulse spacing τ_2. Only 5 of 16 spectra are shown. A central component in the spectra arising from residual HOD is not shown. A 2-kHz Gaussian line-broadening function was applied prior to Fourier transformation.

The spectral densities of motion $J_1(\omega_0)$ and $J_2(2\omega_0)$ can be obtained from the Jeener–Broekaert experiment by measurement of the rate of decay of quadrupolar order (=difference magnetization) and the rate of recovery of the Zeeman order (=sum magnetization). Both processes were observed to be exponential, and J_1 and J_2 were derived from the rate constants according to

$$R_{1Z} = 1/T_{1Z} = \frac{3\pi^2}{2}(e^2qQ/h)^2[J_1(\omega_0) + 4J_2(2\omega_0)] \quad (1a)$$

$$R_{1Q} = 1/T_{1Q} = \frac{9\pi^2}{2}(e^2qQ/h)^2J_1(\omega_0) \quad (1b)$$

For purposes of relaxation calculations, the small deuterium asymmetry parameter can be ignored. The spectral densities of motion, $J_M(\omega)$, are defined as the (one-sided) Fourier transforms of the reorientational time correlation functions, $C_M(t)$, in the laboratory frame:

$$J_M(\omega) = Re\int_0^\infty C_M(t)e^{-i\omega t}\,dt \quad (2)$$

The relaxation times T_{1Z} and T_{1Q} obtained at two Larmor frequencies, $\omega_0 = 38.4$ MHz and $\omega_0 = 76.8$ MHz, are listed in Table I. For comparison, we have included the results for T_{1Z} obtained earlier (Brandes et al., 1986) from randomly oriented calf thymus Li-DNA. Those earlier measurements were made at the perpendicular edges of the nearly axially symmetric power patterns. It is apparent that the two sets of measurements agree quite well, the possible exception being

Table II: Experimental Spectral Densities for Solid Li-DNA

relative humidity (%)	librational amplitudes[a] tilt θ_0 (deg)	twist ϕ_0 (deg)	ω_0 = 38.4 MHz $J_1(\omega_0)$ (ps)	$J_2(2\omega_0)$ (ps)	J_1/J_2	ω_0 = 76.8 MHz $J_1(\omega_0)$ (ps)	$J_2(2\omega_0)$ (ps)	J_1/J_2	frequency dependence J_1^{lo}/J_1^{hi}	J_2^{lo}/J_2^{hi}	J_1^{hi}/J_2^{lo}
35	7 ± 2	10 ± 2	1.2 ± 0.1	0.28 ± 0.06	4.3 ± 1.0						
66	10 ± 2	12 ± 2	6.1 ± 0.3	1.4 ± 0.1	4.4 ± 0.4	2.8 ± 0.1	0.50 ± 0.05	5.6 ± 0.6	2.2 ± 0.1	2.8 ± 0.3	2.0 ± 0.2
75	12 ± 2	13 ± 2	8.6 ± 0.4	2.6 ± 0.3	3.3 ± 0.4	4.2 ± 0.1	0.93 ± 0.08	4.5 ± 0.4	2.0 ± 0.1	2.8 ± 0.4	1.6 ± 0.2
84	15 ± 2	15 ± 2	16.0 ± 1.2	8.4 ± 1.2	1.9 ± 0.3						

[a] Amplitudes of libration were determined (Brandes et al., 1988a) from line splittings in orthogonal orientations of the samples.

the T_{1Z}'s at 84% RH. This agreement is significant, since all different helix orientations are being sampled in the experiments on powders in contrast to those on the aligned samples, where only one helix axis orientation occurs. In powder spectra the situation is further complicated by the fact that at the frequency of the perpendicular edge of one transition 37% of the spectral intensity arises from the other transition and CD bonds making a 35.3° angle with the field. Consequently, if the spin–lattice relaxation rates were strongly anisotropic, the observed recovery should be multiexponential in the powder and would probably appear different from T_{1Z} of the aligned sample. Since the amplitudes of motion in two perpendicular planes are very similar (Brandes et al., 1988b), the fact that the measured rates of recovery are the same for the two samples suggests that the rates of motion in two perpendicular planes are similar, or—in other words—that the librational motion of the CD bonds in reasonably dry DNA samples has nearly uniaxial symmetry.

This conclusion contrasts with the interpretation set forth by Kintanar et al. (1989), who analyzed relaxation data obtained for the purine C_8 deuterons in the dodecamer duplex, $[d(CGCGAATTCGCG)]_2$. In that study the authors ascribed the spin–lattice relaxation at the perpendicular edges of the deuteron powder pattern to a librational motion in a plane perpendicular to the helix axis, simulating the motion by a two-site jump model. The uniaxiality implied by the relaxation data in Table I suggests that this type of motional model does not apply to high molecular weight DNA.

Consideration of the individual spectral densities of motion obtained for the oriented DNA samples confirms this conclusion. $J_1(\omega_0)$ and $J_2(2\omega_0)$ were obtained from eqs 1a and 1b and the relaxation data in Table I. The results are listed in Table II together with some J ratios of diagnostic value. A comparison of the J's at the two fields shows that J_1 is inversely proportional to the Larmor frequency. This ω^{-1} frequency dependence was observed earlier for T_{1Z} in powder samples of both Li- and Na-DNA, and we now see that this dependence is associated with the numerically larger $J_1(\omega_0)$. Although J_2 depends more strongly on frequency, perhaps as $\omega^{-3/2}$, neither the J_1 nor the J_2 frequency dependence is strong enough to be due to diffusive motion, which in the range of relaxation rates encountered here should be proportional to ω^{-2}.

It is clear from the data in Table II that J_1 is much larger than J_2 in all cases. For the three driest samples, the ratio J_1/J_2 falls in the range 3.3–5.6, dropping to 1.9 ± 0.3 at 38.4 MHz and 84% RH. As shown below, provided that the relaxation mechanism is in fact exclusively quadrupolar, such that eqs 1 fully account for the observed relaxation times, this rules out torsional deformation modes or any other motion in a plane perpendicular to the helix axis as the dominant relaxation mechanism in DNA samples hydrated below 84% RH.

Models of Motion. (*A*) *In-Plane Librations.* A full development of appropriate correlation functions for motion in DNA is beyond the scope of this paper. As a minimum a three-step transformation of the deuterium electric field gradient tensor from laboratory to helix axis to base normal to deuterium principal axis system is required, and the restricted motion requires consideration of crosscorrelation as well as autocorrelation terms (R. R. Vold and R. L. Vold, unpublished results). However, if we consider only librational motion of a C_8–D bond in a plane perpendicular to the base pair normal, then the treatment can be simplified, and the spectral densities appearing in eqs 1 can be written as

$$J_M(M\omega_0) = \sum_K \sum_{K'} d^{(2)}_{MK}(\beta) d^{(2)}_{MK'}(\beta) e^{i\gamma(K-K')} d^{(2)}_{K0}(\beta') d^{(2)}_{K'0}(\beta') \times \int_0^\infty [\Gamma_{KK'}(t) - \langle e^{-iK\gamma(0)}\rangle \langle e^{iK'\gamma(t)}\rangle] e^{iM\omega_0 t}\, dt \quad (3)$$

where $\Gamma_{KK'}(t) = \langle e^{-iK\gamma(0)} e^{iK'\gamma(t)}\rangle$ is the reorientational correlation function describing the librational motion about the base normal. The precise form of $\Gamma_{KK'}(t)$ depends on the specific model of torsional motion, which can be modeled as discrete jumps (Shindo et al., 1987; Kintanar et al., 1989), restricted diffusion on a ring (London & Avitabile, 1978), or collective torsional modes (Allison & Schurr, 1979; Barkley & Zimm, 1979). The $d^{(2)}_{ij}$ are the reduced second-rank Wigner rotation elements, functions of either the polar angle β, which determines the orientation of the base normal in the laboratory frame, or of the polar angle β' between the base normal and the principal axis of the electrical field gradient of the purine 8-deuteron. β' is obviously equal to 90°, and β includes both the angle between the base normal and the helix axis and the angle between the helix axis and the magnetic field. The two terms in angular brakets, $\langle\ \rangle$, refer to time averages over the accessible range of the angle γ, which defines the position of one CD bond on a circle about the base normal. These averages are independent of time and will in general be different from zero. However, it can be shown that these terms are exactly canceled by the long term limit of $\Gamma_{KK'}(t)$, thereby allowing normal Fourier transformation of the quantity inside the square brackets.

Equation 3 contains 25 terms for each value of M. The terms with K and K' equal to zero do not contribute to relaxation, and for $\beta' = 90°$ the expression for $J_M(M\omega_0)$ reduces to just a few terms. Further reduction of eq 3 occurs when the angle β is small. This applies in the present situation, where the DNA sample is oriented with the helix axis parallel to the field. In fact, the angle β includes contributions from base tilt, roll, and/or propellering, but it is normally less than ca. 22°. Under conditions of small β one can show (R. R. Vold and R. L. Vold, unpublished results) that only the autocorrelation function Γ_{22} contributes to the $C_M(t)$ and that, to second order in β, the following relation holds between the two correlation functions of interest:

$$C_1(t)/C_2(t) = \beta^2 \quad (4)$$

Thus, for $\beta \leq 22°$, $C_1(t)/C_2(t) \leq 0.14$. Since the spectral densities probed in a single relaxation experiment are different

Table III: Spectral Densities of Motion Calculated from the Diffusion in a Cone Model

relative humidity (%)	cone half-angle (deg)	38.4 MHz		76.8 MHz		correlation time, τ_c (μs)
		$J_1(\omega_0)$ (ps)	$J_2(2\omega_0)$ (ps)	$J_1(\omega_0)$ (ps)	$J_2(2\omega_0)$ (ps)	
35	7	1.2	0.30	0.30	0.08	3.1
66	10	7.5	1.9	1.90	0.48	0.48
75	12	11.8	3.0	3.0	0.76	0.30
84	15	17.1	4.4	4.4	1.21	0.20

Fourier components of $C_1(t)$ and $C_2(t)$, a similar simple relationship does not hold between $J_1(\omega_0)$ and $J_2(2\omega_0)$. Nevertheless, at this sample orientation the ratio J_1/J_2 will always be substantially less than one for torsional motion about the base normal, regardless of the type or rate of this motion. As shown in Table II, this is in direct contrast to what is observed experimentally.

When spectral densities have been measured at two fields, one twice as high as the other, concerns about the trivial frequency dependence can be eliminated by comparing $J_1(\omega_0)$ at the higher field with $J_2(2\omega_0)$ at the lower field (Vold & Vold, 1979; Vold, R. R., et al., 1981). We have included this ratio, J_1^{hi}/J_2^{lo}, as the last column in Table II. The ratio is seen to be considerably larger than 1, showing unequivocally that torsional motion about the base normal is insignificant in causing relaxation at the three lowest degrees of hydration investigated here. One should note, however, that all J_1/J_2 ratios decrease at 84% RH, probably indicating that contributions to relaxation from in-plane motion are on the increase.

(*B*) *Diffusion in a Cone.* In order to account for the large values of J_1/J_2, it is necessary to include motion with components out of the plane, as already suggested by the similarities between the T_{1Z}'s for the two kinds of samples. Although eq 3 no longer applies and the algebra is tedious, this can be done by considering the internal motion as either simple uniform restricted diffusion (London & Avitabile, 1978) or overdamped rotations in a harmonic potential (Schurr, 1982) in two orthogonal planes. In view of the nearly uniaxial nature of the motion, a simpler expedient is to treat the local motion as diffusion in a cone (Wang & Pecora, 1980; Lipari & Szabo, 1981). While the physical reality behind these models is different, the formal solutions and the resulting values of the correlation times are quite similar. Diffusion in a cone has been used previously to interpret NMR relaxation times in anisotropic media (Selwyn et al., 1984; Brandes et al., 1986). The relevant spectral densities of motion in the laboratory frame consist of sums of Lorentzians:

$$J_M(M\omega_0) = \sum_N [d^{(2)}_{MN}(\beta)]^2 J_N(M\omega_0) \qquad (5)$$

where the spectral densities $J_N(M\omega_0)$ in the frame of the cone are given by

$$J_N(M\omega_0) = c_N \sum_i a^i_N \frac{b^{(i)}_N \tau_c}{1 + (M\omega_0)^2 (b^{(i)}_N \tau_c)^2} \qquad (6)$$

Here the correlation time, τ_c, is defined to be that of unrestricted rotational diffusion with $\tau_c = 1/6D$. This familiar correlation time is modified by the $b^{(i)}_N$, the inverse of the eigenvalues of the diffusion equation for diffusion in a cone, while the $a_N(i)$ are obtained from the eigenvectors and the c_N are normalization factors (Wang & Pecora, 1980). For cone angles less than ca. 60°, Wang and Pecora, (1980) showed that one term in the sums dominates, and approximate expressions have been provided for this situation (Lipari & Szabo, 1981).

As pointed out before (Brandes et al., 1986), fits of this model to deuterium relaxation data for partially hydrated DNA are not satisfactory, but they can at least provide an estimate of the time scale of the motion. Spectral densities of motion, calculated from eqs 5 and 6 to fit the experimental data, are listed in Table III together with the best estimates of the correlation times for the cone motion. In calculating these correlation times we assumed that the motion was in the "slow-motion" limit to take into account the observed frequency dependence as well as the observation of faster relaxation at a higher temperature (Brandes et al., 1986). We consequently report correlation times that are several orders of magnitude larger than that, 85 ps, reported by Kintanar et al. (1989) for the dodecamer at 66% RH. However, the values reported in the two studies are largely incommensurate, their values being a measure of the rate of jumping between two sites and our characterizing a diffusive process over a different domain. For the cone model, the effective correlation times, $b^{(i)}_N \tau_c$, would be more directly comparable to those reported by Kintanar et al. (1989). Although they vary with the value of N, for cone angles of 7–15° the values of $b^{(i)}_N \tau_c$ range from ca. $0.01\tau_c$ to $0.05\tau_c$. For the data in Table III this would correspond to effective correlation times in the range 10^{-9}–10^{-8} s, still considerably longer than reported for the dodecamer (Kintanar et al., 1989).

(*C*) *More Complex Models of Motion.* We have explored whether fits to our experimental data could be improved by considering a more general model developed originally to explain deuterium relaxation in thermotropic liquid crystals (Vold & Vold, 1988). This approach allows motion of the base normal to be described by two diffusion constants instead of one and combines diffusional motion of the base normal with torsional deformation modes. The fits show no improvement over those obtained with the simple cone model, since the observed frequency dependence is consistently less strong than that calculated from any of these models. Possibly, one way to obtain better agreement with experimental data could be to include collective base motion with out-of-plane components, such as the bending modes of Barkley and Zimm (1979) or to develop an alternative model similar to those used in liquid crystals (Blinc, 1976) for coherent motion of the nematic director.

An Estimate of Possible Relaxation Contribution from Protons. No discussion of the deuterium relaxation as a function of water content would be complete without addressing the question: Do dipolar interactions with protons contribute at all? While an accurate calculation of these contributions is not practical, a useful estimate can be made by treating the (anisotropic) motion of each H–D dipolar tensor as diffusion in a cone.

Heteronuclear dipolar interactions contribute to the deuterium relaxation rate only through the spectral density $J_1(\omega)$. They are orientation dependent, but if we ignore any dependence of the relaxation rate upon cone orientation (which would reduce the effect), the dipolar contribution from protons can by analogy with eq 6 be written

$$R_1(\mathrm{DH}) = \frac{N}{2}\gamma_H^2\gamma_D^2\hbar^2 r^{-6}\tau_c L = (6.715 \times 10^{-39}) r^{-6} N \tau_c L \qquad (7)$$

with

$$L = \frac{a_0 b_0 c_0}{1 + (\omega_H - \omega_D)^2 (b_0 \tau_c)^2} + \frac{3 a_1 b_1 c_1}{1 + \omega_D^2 (b_1 \tau_c)^2} + \frac{6 a_2 b_2 c_2}{1 + (\omega_H + \omega_D)^2 (b_2 \tau_c)^2} \quad (8)$$

Here N is the number of nearby protons, r is the proton–deuteron distance, and, as in eq 6, a_M, b_M, and c_M are the cone coefficients that depend only on cone angle. At 84% RH the librational amplitude of the C–D bond motion is 15°; thus for a proton bound to DNA, 15° may be used as an estimate of the maximum amplitude for the relative motion of the H–D pair. For this cone angle, $L\tau_c$ turns out to go through a maximum at $\tau_c = 3.5 \times 10^{-8}$ s. If, as a worst case scenario, we let $N = 10$ and all $r = 2$ Å, we then find a minimum value of T_1(HD) = 3.0 s. This is not much longer than the value of T_1 observed at 35% RH. However, at that low humidity the motional amplitude is much smaller, and the dipolar interaction would decrease drastically, regardless of the number of nearby protons. An estimate of the contributions from mobile H_2O molecules is much less certain, since the internuclear *distance* as well as angle fluctuates. Nevertheless, we consider it safe to rule out major dipolar contributions to the deuterium relaxation rate.

Summarizing Remarks

The present determination of a set of spectral densities of motion for partially hydrated samples of oriented Li-DNA has confirmed the interpretation of earlier deuteron relaxation measurements on randomly oriented DNA (Brandes et al., 1986): as a hydration level is increased, the amplitude and rate of librational motion increase with a resulting drop in the observed relaxation times. In the hydration range explored here (35–84% RH), we have now found $J_1(\omega_0)$ to be consistently greater than $J_2(2\omega_0)$ when the DNA is aligned with the helix axis parallel to the magnetic field. This observation proves that torsional motion about the helix cannot be the dominating relaxation mechanism, regardless of the type and rate of this torsional motion. Closer inspection of the observed trends in the spectral densities as the hydration increases does indicate, however, that (slower) torsional motion of increasing amplitude should become significant as the hydration level is increased about 84% RH.

An analysis of the spectral densities of motion in terms of a simple diffusion in a cone model does not fit the spectral densities very well either. In particular, the experimentally observed frequency dependence is weaker than that predicted from a diffusion model. Nevertheless, the model allows us to make an estimate of the rotational correlation times, which fall in the range 0.2–3.0 μs.

It is clearly important to continue the search for better models of the internal motion in DNA, probably combining elastic deformation (bending) modes with local, random motion. It is also essential to verify experimentally that our neglect of dipolar contributions to the relaxation rates is justified. Furthermore, relaxation studies of DNA at higher humidities should be continued. Concerns for sample integrity and heavy loss of signal to noise have unfortunately so far made accurate measurements of the spectral densities of motion impossible for oriented samples at high humidity. It is our hope that the anisotropy of relaxation rates in deuterium powder patterns of heavily hydrated samples, combined with line-shape analyses similar to those reported earlier (Shindo et al., 1987), can be used instead to probe the internal motion in solid DNA.

Acknowledgments

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