Journal of Biomolecular NMR, 8 (1996) 77–86
ESCOM

J-Bio NMR 357

Water molecule binding and lifetimes on the DNA duplex d(CGCGAATTCGCG)$_2$

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Received 12 September 1995
Accepted 30 March 1996

Keywords: Water; DNA dodecamer; DNA binding; d(CGCGAATTCGCG)$_2$; Water–DNA lifetimes

Summary

Measurements of the water proton spin-lattice relaxation rate for aqueous solutions of the palindromic dodecamer, d(CGCGAATTCGCG)$_2$, are reported as a function of the magnetic field strength. The magnitude of the relaxation rates at low magnetic field strengths and the shape of the relaxation dispersion curve permit assessment of the number of water molecules which may be considered bound to the DNA for a time equal to or longer than the rotational correlation time of the duplex. The data are examined using limiting models that arbitrarily use the measured rotational correlation time of the polynucleotide complex as a reference point for the water molecule lifetime. If it is assumed that water molecules are bound at DNA sites for times as long as or longer than the rotational correlation time of the duplex, then the magnitude of the relaxation rates at low field require that there may be only two or three such water sites. However, if the lifetime constraint is relaxed, and we assume that the number of water molecules bound to the DNA is more nearly the number identified in the X-ray structures, then the average water molecule lifetime is on the order of 1 ns. Measurements of ^{1}H NOESY spectra demonstrate that some water molecules must have lifetimes sufficiently long that negative Overhauser effects are observed. Taken together, these results suggest a distribution of water molecule lifetimes in which most of the DNA-bound water molecule lifetimes are shorter than the rotational correlation time of the duplex, but where some have lifetimes of at least 1 ns under these concentrated conditions.

Introduction

Water interactions have been of long standing interest in attempts to understand macromolecular energetics and dynamics. A variety of NMR methods have demonstrated that the water molecules at a number of surfaces including proteins (Shirley and Bryant, 1982; Polnaszek and Bryant, 1984), silica gel (Polnaszek et al., 1987), and phospholipids (Whaley et al., 1994), are characterized by rapid local motion and translational diffusion coefficients that are within a factor 10 of bulk water values (Kimmich et al., 1990; Kotitschke et al., 1990). Recent reports based on high-resolution 2D NOE measurements between water protons and protein protons have demonstrated that there are a few unique water-molecule binding sites which have measurable magnetization transfer with the protein but water lifetimes that are short compared with approximately 10^{-4} s (Otting and Wüthrich, 1989; Otting et al., 1991a–c). The vast majority of water molecules in contact with the protein do not display a significant nuclear Overhauser effect and thus have lifetimes on the surface that are estimated to be short compared with 300 ps. This conclusion is consistent with a number of magnetic relaxation studies of water adsorbed on protein molecules, which conclude that the water at the protein surface is characterized by local correlation times in the hundreds of ps (Shirley and Bryant, 1982; Kimmich et al., 1986, 1990; Schauer et al., 1988; Kimmich, 1990). The high-resolution approaches have been extended to DNA, i.e., the title compound (Kubinec and Wemmer, 1992; Liepinsh et al., 1992), which is a good model because the self-complimentary dodecamer duplex is extensively studied in the solid state (Dickerson and Drew, 1981; Drew et al., 1981, 1982; Fratini et al., 1982; Kopka et al., 1983; Wing et al.,

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Abbreviations: DNA, deoxyribonucleic acid; NOE, nuclear Overhauser enhancement; NOESY, nuclear Overhauser enhancement spectroscopy.

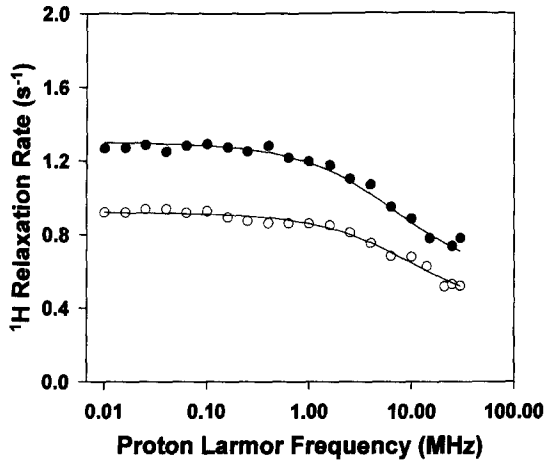

Fig. 1. The water proton spin-lattice relaxation rate as a function of the magnetic field strength reported as the proton Larmor frequency for 6.4 mM aqueous solutions of d(CGCGAATTCGCG)$_2$ at pH 7, at (○) 278 K and (●) 288 K. The solid lines were computed according to Eq. 3.

1985; Quintana et al., 1991; Edwards et al., 1992), and the modest molecular weight permits efficient application of high-resolution NMR methods. The free oligomer forms a B-DNA helix in the crystal, a structure that is consistent with one- and two-dimensional NMR studies in solution (Pardi et al., 1982; Patel, 1982; Patel et al., 1982,1983; Hare et al., 1983; Rajagopal et al., 1988; Thomas et al., 1989). The Dickerson proposal that the zig-zag spine of water in the minor groove is responsible for stabilizing the B-form of the DNA structure makes these water molecules of particular interest both dynamically and thermodynamically (Kopka et al., 1983). The Wüthrich and Wemmer groups have reported 2D NOE measurements that identify several binding sites in the minor groove (Kubinec and Wemmer, 1992; Liepinsh et al., 1992). We report here magnetic relaxation dispersion measurements that permit characterization of the water molecule lifetimes in terms of limiting models. The dynamical results for the DNA duplex are similar to those found for proteins to date: either there are very few water molecules bound to the DNA for times as long as the rotational correlation time for the DNA, of order 25 ns under these conditions, or, on average, the distribution of water molecule lifetimes must be heavily weighted at times short compared with 25 ns.

Materials and Methods

The DNA dodecamer, d(CGCGAATTCGCG)$_2$, was obtained from the Midland Certified Reagent Company (Midland, TX) as the lyophilized material and the sample purity checked by NMR spectroscopy. ^{1}H nuclear magnetic relaxation dispersion measurements were obtained from solutions supported with 20 mM sodium phosphate buffer at pH 7.0 in 10 mM sodium chloride, containing 8.2 mM DNA duplex based on the optical absorption at 260 nm with an extinction coefficient of 1.935×10^5 $M^{-1}cm^{-1}$ for the duplex (Ott and Eckstein, 1985). Samples for ^{1}H spectroscopy at 500 MHz contained 5.07 mM DNA duplex, 20 mM sodium phosphate, and 10 mM sodium chloride in 80% H_2O and 20% D_2O with a pH meter reading of 7.0. Paramagnetic metal ion contamination would substantially increase the low-field relaxation rates and generally show a characteristic magnetic field dependence at higher field strengths that was not observed.

The two-dimensional NMR measurements were made on a Varian Unity Plus Spectrometer operating at a proton resonance frequency of 500 MHz. The 2D NOESY spectra were recorded using a pulse sequence adapted from Smallcombe (1993), for observing exchangeable protons in combination with the SUPERB-W selective excitation technique, which is a family of small flip-angle pulses that simultaneously suppress a solvent resonance while producing nearly uniform phase excitation over other regions of the spectrum (Fetler et al., 1993). With this approach we were able to suppress the water signal by a factor of several thousands without saturation and retain good phase response over approximately 75% of the spectrum. Pulsed magnetic field gradients were used along the dc magnetic field direction during the mixing period to eliminate the need for full phase cycling. The 2D NOESY spectra were obtained in pure absorption phase to minimize artifacts caused by single- and double-quantum coherences and suppresses the effects of zero-quantum coherence (States et al., 1982; Gerothanassis, 1994). A spectral width of 9600 Hz was used to collect 16 transients at each of 384 t_1 values. Gaussian line broadening of 3 Hz was applied in both dimensions, and data were zero-filled to 2048 points in the t_1 dimension prior to Fourier transformation.

Water proton spin-lattice relaxation rates were measured using a field-cycling NMR spectrometer described elsewhere (Redfield et al., 1968; Hernandez et al., 1990). The temperature of the sample was regulated by a recirculating bath of perchloroethylene controlled by a Neslab RTE-8 temperature controller and outboard Little Giant pumps. The statistical errors for the relaxation rates are estimated at 3%.

Results

The water spin-lattice relaxation rates for aqueous DNA solutions are shown as a function of the magnetic field plotted as the proton Larmor frequency in Fig. 1. There are two notable features: (i) there is a readily detected dispersion similar to that observed in protein cases, though the inflection is at relatively high frequency compared with larger globular proteins; and (ii) the amplitude of the dispersion is small, i.e., the difference be-

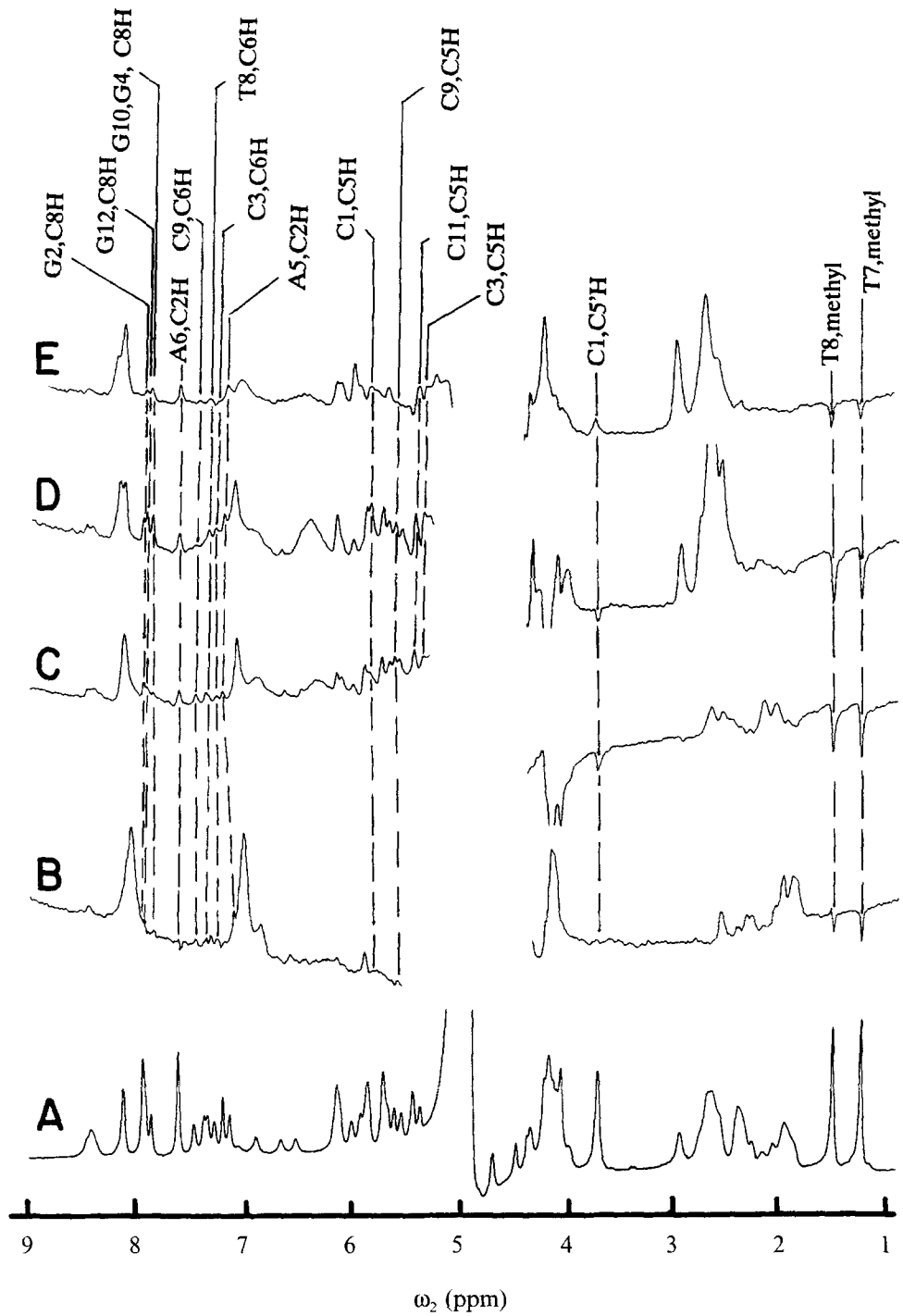

Fig. 2. Proton spectra obtained at 500 MHz for a 3.9-mM DNA duplex in 80% H_2O and 20% D_2O, at a pH meter reading of 7.0 at several temperatures. (A) The one-dimensional spectrum with the presaturation pulse sequence at 283 K; (B) a cross section through the NOESY two-dimensional spectrum with a mixing time of 50 ms at the water resonance in the ω_1 direction at 298 K; (C) the NOESY cross section as in (B), but at 289 K; (D) the NOESY cross section as in (B), but at 283 K; (E) the NOESY cross section as in (B), but at 278 K.

tween the low-field and high-field relaxation rates is not very large, even though the DNA concentration is 6.4 mM.

NOESY experiments were performed at 278, 283, 289, and 298 K using different mixing times, and Fig. 2 collects cross sections through the spectra of the DNA duplex at the chemical shift of the water in the ω_1 direction obtained with a mixing time of 50 ms. The chemical shift range from 12 to 14 ppm is not shown, because this area is dominated by imino proton resonances that exchange with the water protons directly and provide chemical exchange peaks (Pardi et al., 1982). Nonlabile proton

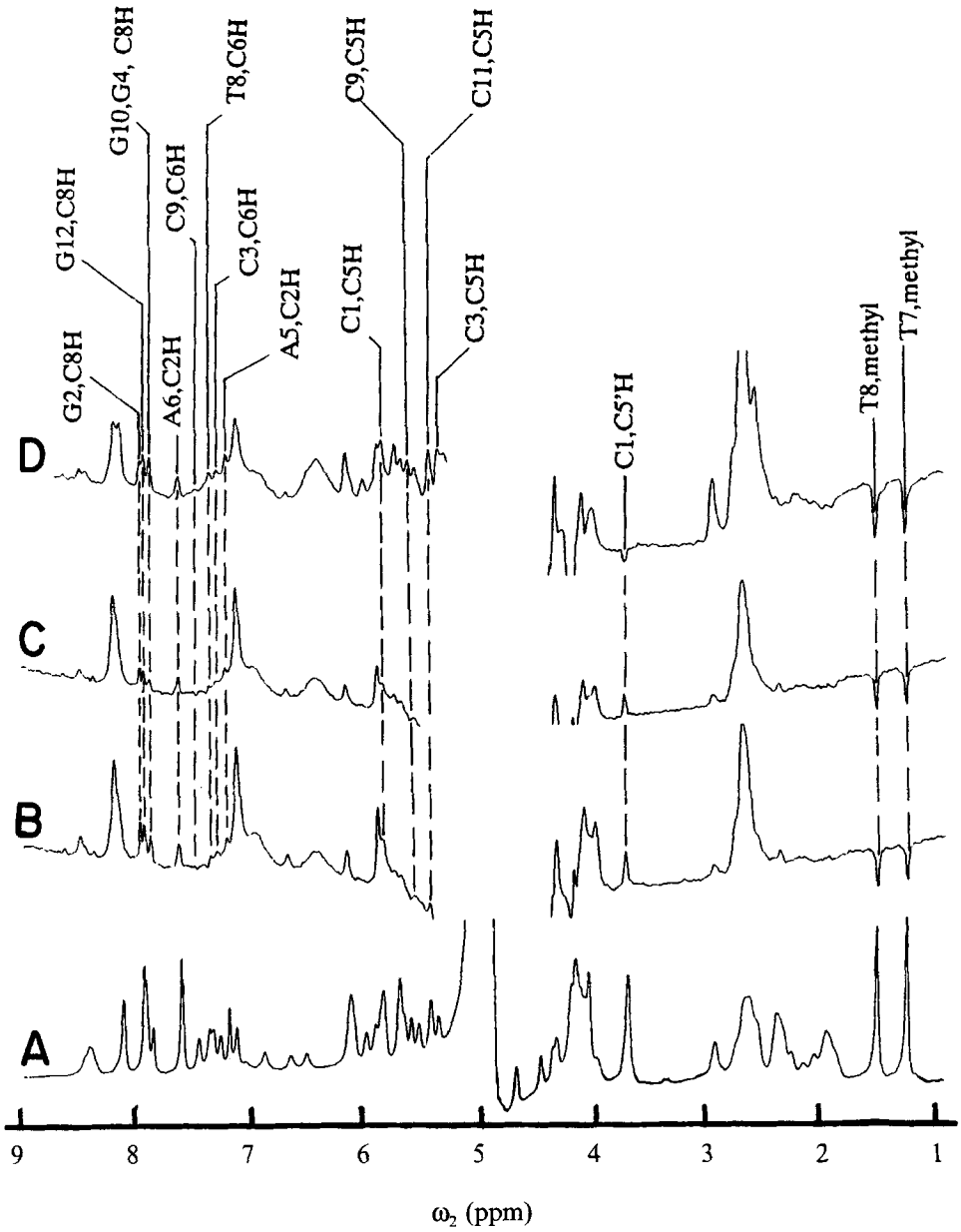

Fig. 3. Proton spectra obtained at 500 MHz for a 3.9-mM DNA duplex in 80% H_2O and 20% D_2O, at a pH meter reading of 7.0, at 283 K. (A) The one-dimensional spectrum obtained with the water-suppression pulse sequence; (B) the cross section through the NOESY spectrum as in Fig. 2, but with a mixing time of 180 ms; (C) the NOESY cross section as in (B), but with a mixing time of 100 ms; (D) the NOESY cross section as in (B), but with a mixing time of 50 ms.

assignments follow the results of Hare and co-workers (1983) and the labile proton assignments follow the results of Rajagopal et al. (1988) and Sklenář et al. (1987). The cross-peak intensities are summarized along with the assignments for cross-peaks to the base protons in Table 3 and for the deoxyribose protons in Table 4. The labeling convention is that the C terminus is nucleotide 1 and the G terminus is nucleotide 12. The proton-numbering convention is conventional. As pointed out by Turner (1985), positive cross-peaks in the NOESY spectrum correspond to negative nuclear Overhauser effects.

The NOESY spectra cross-peak intensities are a function of the mixing time, as shown in Fig. 3. We focus attention on the short mixing times because of possible complications from spin diffusion, but include the longer mixing time data for comparison.

Discussion

Magnetic relaxation dispersion

Magnetic relaxation dispersion provides a direct approach to characterization of water dynamics in macromolecular solutions. The relaxation data in Fig. 1 show clearly that there is an effective magnetic coupling be-

tween the water molecule spin-lattice relaxation rate and the rotational motion of the DNA molecule. The problem of the coupled relaxation may be treated conveniently using the solution for the longitudinal magnetization for two spin populations discussed originally by Solomon (1956). The extension to spin populations has been discussed by many (Kalk and Berendsen, 1976; Edzes and Samulski, 1977,1978; Koenig et al., 1978; Eisenstadt, 1980, 1985; Fung and McGaughy, 1980; Shirley and Bryant, 1982). The essence of the model is that although there are many sites on the DNA which may interact with water, there is no resolution available in the water resonance to measure each contribution. The pseudo-first-order rate constant in Eq. 1 then represents the sum over the relaxation contributions from all sites. Since the relaxation times measured are larger than 0.1 s, there is little likelihood that the averaging process implied by this approximation is incomplete. The effective averaging of all water binding site contributions is further justified by both the water relaxation data reported here and the nuclear Overhauser effect data.

The coupled differential equations for the response of the two populations generally yield a bi-exponential recovery to equilibrium. However, if one population (the water in this case) is in large excess, only a single decay time is detectable. The magnetic relaxation experiments reported here were performed with an instrument that switches the magnetic field rapidly, but not sufficiently rapidly that the shorter relaxation components may be detected. The slow relaxation component, R_s, is given by:

$$R_s = \frac{1}{2}\left[R_W + R_M + R_{WM}\left(1+\frac{1}{F}\right) - \left[\left[R_M - R_W - R_{WM}\left(1-\frac{1}{F}\right)\right]^2 + \frac{4R_{WM}^2}{F}\right]^{0.5}\right] \quad (1)$$

where R_W is the water proton spin-lattice relaxation rate, R_M the spin-lattice relaxation rate of the protons associated with the macromolecule, R_{WM} the pseudo-first-order magnetization transfer rate, and F is the ratio of the macromolecule and bound water proton population to the bulk water proton population. In the limit that the magnetization transfer rate is rapid and F is small, Eq. 1 reduces to the usual rapid chemical exchange model:

$$R_S = R_W + FR_M \quad (2)$$

The value of R_W is independent of magnetic field strength over the values studied and equal to 0.33 s^{-1} at 298 K when the sample is in equilibrium with atmospheric oxygen and has a proton mole fraction of one. The essence of the development to follow is that we apply a limiting and elementary model to the interpretation of R_M suggested by the X-ray structure reported for the water molecules on the DNA structure, but which leads to an inconsistency. The resolution of the inconsistency requires modification of a primary and common assumption about the lifetimes of the water molecules on the DNA structure.

The water proton spin-lattice relaxation rate may be coupled to the motion of the DNA molecule by three mechanisms: (i) a dipole–dipole coupling between the water protons and the DNA protons made time-dependent by translational motion of the water near the DNA molecule; (ii) chemical exchange of protons from ionizable groups of the macromolecule to the bulk water molecule pool; and (iii) exchange of bound whole water molecules with the bulk water molecule pool. The translational correlation times for water near macromolecules have been measured by several approaches, and the correlation times are generally less than a factor 10 longer than in the bulk solution (Polnaszek and Bryant, 1984a,b; Polnaszek et al., 1987; Kimmich et al. 1990; Kotitschke et al., 1990). Therefore, we neglect the translational contribution because the very short correlation time for the dipole–dipole coupling should make it small compared with the other contributions. The contributions from proton transfer are generally a function of the folded structure of the macromolecule, the pH, proton exchange catalysts, and temperature. For the purposes of this discussion, we neglect this contribution but recognize that this neglect may overestimate the remaining contribution. Therefore, we explore the consequences of the assumption that the magnitude of the water proton relaxation rate observed depends only on exchange of whole water molecules with bound sites on the DNA molecule.

The consequence of these assumptions is now that the fast-exchange condition leading to Eq. 2 corresponds to the assumption that the exchange of water molecules between the DNA sites and the liquid is fast compared with the relaxation rate, R_M. We address the validity of this assumption later. The magnetic field dependence of R_M has been modeled in several ways. In this solution case, DNA rotates diffusively so that Lorentzian spectral densities are expected. Any time the reorienting macromolecule is not spherical, the reorientational motion may be characterized by more than one reorientational correlation time. If these correlation times are significantly different, more than one inflection point in the relaxation dispersion curve will result, which is not apparent in the data of Fig. 1. However, the effect of anisotropy in the rotational diffusion of the macromolecule is to broaden the relaxation dispersion curve. Generally, macromolecular spectral densities that are mapped onto the water spin relaxation by cross-relaxation appear to be broadened, i.e., not be a simple Lorentzian (Koenig and Schillinger, 1969; Koenig and Hallenga, 1976). Contributions to this broadening may include local motion in the macromolecule environment, which produces a distribution of effec-

tive correlation times, rapid chemical exchange of bound or buried water molecules which interrupts the rotational motion in the bound site, and chemical exchange of labile protons on the macromolecule. Since the evaluation of the effective correlation time for the bound environment is crucial to the quantitative aspects of the argument, we assume two different models for the magnetic field dependence of the spin-lattice relaxation rate, and then compare them to show that the details of the model for the bound site relaxation make very little difference to the dynamical conclusions drawn from the data.

As one model we assume a Cole–Cole function to characterize the magnetic field dependence of the DNA site relaxation, a choice that is partially justified by the rotational character of the problem (Cole and Cole, 1941). In this case:

$$R_M = A\left(\frac{1+(2\pi\nu\tau_c)^{\beta/2}\cos(\pi\beta/4)}{1+2(2\pi\nu\tau_c)^{\beta/2}\cos(\pi\beta/4)+(2\pi\nu\tau_c)^{\beta}}\right) \quad (3)$$

where ν is the proton Larmor frequency, τ_c the correlation time for the dipole–dipole interaction, and A and β are adjustable parameters.

Another model developed by Kimmich and co-workers based on defect diffusion as a reorientational mechanism is:

$$R_M = 2A\sqrt{\pi}\cos(B\arctan(2\pi\nu\tau_c))\frac{\tau_C^B}{(1+4\pi^2\nu^2\tau_c^2)^{\beta/2}} \quad (4)$$

where A and B are parameters of the model, ν the Larmor frequency, and τ_c the correlation time for the dipole–dipole coupling (Kimmich and Doster, 1976; Kimmich and Winter, 1985). In highly hydrated protein gels, B is found to be 0.5, corresponding to two-dimensional defect diffusion (Zhou and Bryant, 1994).

For each model we temporarily make the critical assumption that the correlation time for the dipole–dipole interaction that relaxes the water molecule proton spins is the rotational correlation time for the DNA molecule. This assumption requires that the water molecules that are exchanging with the liquid, and carry the magnetic relaxation from the DNA to the bulk liquid, experience the DNA binding site for a time long compared with the rotational correlation time of the DNA molecule. Furthermore, in the bound environment, we neglect the contributions of any DNA protons to the water spin-lattice relaxation rate. On the average, the distance between a water proton and the nearest DNA protons will be 2.2 Å or larger while the intramolecular water proton distance is 1.507 Å. Thus, the contribution from the other water proton will be more than seven times as efficient as the next nearest neighbor. Therefore, we approximate the bound water molecule relaxation rate by the intramolecular contribution only; i.e., the relaxation of the water proton caused by the other water molecule proton where the bound water molecule is assumed to rotate with the correlation time of the DNA duplex. This assumption that relaxation is dominated by intramolecular effects is known to be false in at least some cases, because there are observable water-proton to DNA-proton nuclear Overhauser effects. However, this neglect causes the model to underestimate the relaxation rate of the water molecule bound to the DNA molecule. Since we will use this strategy to estimate the number of bound water molecules, this assumption will lead to an overestimate for the number of water molecules bound to the DNA for times as long as the rotational correlation time of the DNA.

In summary, we assume that relaxation of water protons when bound to the DNA sites is caused by intramolecular water-proton–water-proton dipole–dipole interactions. If we know the rotational correlation time of the DNA, which we may extract from the relaxation dispersion profile of Fig. 1 using either Eq. 3 or Eq. 4 above, then we may estimate the number of water molecules bound from the magnitude of the relaxation rate at low magnetic field strength. We write the parameter F as:

$$F = n\,[DNA]/[H_2O] \quad (5)$$

where n is the number of water molecules bound to the DNA for a time as long as, or longer than, the rotational correlation time of the DNA molecule; the concentrations are known.

The intramolecular contribution to the water proton relaxation time in pure water has been discussed in some detail and is estimated at 0.16 to 0.18 s^{-1} with the rotational correlation time of 2.5 ps at 298 K (Krynicki, 1966; Hertz, 1967; Goldammer and Zeidler, 1969). We use just the intramolecular relaxation rate, because the model for the bound state relaxation is water-proton–water-proton dipole–dipole coupling only. The rotational correlation times for the DNA complex are summarized in Table 1 for the Cole–Cole model and in Table 2 for the Kimmich model for the magnetic field dependence of the relaxation rates. We compute the value of the bound water relaxation rate as:

$$R_M = 0.18\,s^{-1}\frac{\tau_{c,\,bound}}{\tau_{c,\,water}} = 0.18\,s^{-1}\frac{\tau_{c,\,bound}}{2.5\,ps} \quad (6)$$

Substitution of this result into the rearranged Eq. 2 gives:

$$n = \frac{(R_S - R_W)[H_2O]}{R_M\,[DNA]} \quad (7)$$

The value obtained for n is clearly a sensitive function of the value used for the correlation time; thus, we compare two models. The values for n obtained using both models

TABLE 1
NUCLEAR RELAXATION DISPERSION FOR THE COLE–COLE MODEL

Temperature	A (s^{-1})	β	τ_c (ns)	n(/DNA)	R_W (s^{-1})
278 K	0.754	1.367	23.0	2.89	0.55
288 K	0.533	1.405	17.5	2.81	0.39

for the relaxation dispersion are summarized in Tables 1 and 2. The accurate evaluation of the rotational correlation time is weakly model-dependent, as shown by the different entries in Tables 1 and 2. In this context, it is interesting to compare the value for the rotational correlation time obtained with that for a different but similar dodecamer using light-scattering methods. Measurements on the $((GC)_6)_2$ molecule yield a rotational correlation time of 6.6 ns, which when brought to the temperature of this study becomes approximately 7.5 ns (Haber-Pohlmeier and Eimer, 1993). The values of the correlation time in Tables 1 and 2 are larger suggesting that there is some aggregation. Light-scattering and NMR studies on the dodecamer used in the present study also have reported rotational correlation times that are shorter by as much as a factor of three than the present data suggest (Eimer et al., 1990; Nuutero et al., 1994). The concentrations of DNA used in these studies are very high and aggregation is likely (Nuutero et al., 1994); however, aggregation does not change the arguments because the magnetic relaxation dispersion experiment reports both the strength and the correlation time for the relaxation coupling. This measured value for the correlation time is used as a time marker for the water lifetime conclusions. If the rotational correlation time were too large for a factor of three, the value of n derived would be too small by a factor of three. The resulting number of long-lived water molecules is still much smaller than the number identified in the X-ray structures, and the conclusions are not significantly changed. Since these experiments represent a measurement of the spectral density function over a significant range of Larmor frequencies, large errors in the correlation time appropriate for these samples are unlikely.

To reach the estimates for the number of bound water molecules, the spirit of the model was to neglect chemical exchange of protons between water and ionizable groups on the DNA, such as OH or NH. It is possible that such exchange events carry a significant amount of the relaxation coupling. If this contribution were included in the analysis, the effect would be to reduce the size of n further. An attempt to include additional intermolecular cross-relaxation contributions to the estimate of the bound water molecule relaxation rates, including the effects of adventitious metals, will make the effective R_M larger and the derived value of n smaller by as much as a factor of two or more, while errors in the relaxation rates and concentrations may add a random error of approximately 10%. There may be contributions from exchange of labile protons on the DNA to the observable relaxation rate of the water protons that will have two effects: (i) the inflection frequency for the relaxation dispersion from this contribution will be the rotational correlation time for the DNA, because the lifetimes of these protons are longer than the rotational correlation time by orders of magnitude; and (ii) the magnitude of the low-field relaxation rate will be increased by this contribution, which will increase the estimate of the number of water molecules bound by Eq. 7.

The several assumptions made to reach these estimates all lead to an underestimate of R_M, which causes an overestimate for the number of bound water molecules by at least a factor of two. Nevertheless, both models used to extract a rotational correlation time from the relaxation dispersion profile yield a number of bound water molecules that is small compared with the number identified from the X-ray scattering data. This result does not mean that the water molecules are not there; it does mean that the critical assumption that the lifetimes of the bound water molecules are as long as the rotational correlation time of the DNA duplex cannot be correct for all water molecules identified by the diffraction studies. Based on these relaxation data we are faced with the following choice: either the number of water molecules bound to the DNA duplex is very small, on the order of three or less per duplex, or most of the bound water molecules have lifetimes at the DNA sites that are short compared with the rotational correlation time of the duplex.

This development has permitted estimates of the maximum number of what might be characterized as irrotationally bound water molecules, which appears to be small. Rotation about a single hydrogen-bound axis similar to a methyl-group rotation would decrease the estimate of R_M by approximately a factor of three and increase the estimate of n by the same amount (Marshall, 1970; Marshall et al., 1972). Furthermore, we have assumed until this point that all bound water molecules are the same. It is more reasonable to assume that different

TABLE 2
NUCLEAR RELAXATION DISPERSION FOR THE KIMMICH MODEL

Temperature	A ($s^{-(1+B)}$)	B	τ_c (ns)	R_W (s^{-1})	F	n(/DNA)	R_{WP} (s^{-1})
278 K	720 000	0.52	32	0.55	0.00307	2.08	69
288 K	540 000	0.52	26	0.39	0.00307	1.89	56

TABLE 3
NOE CROSS-PEAKS BETWEEN WATER PROTONS AND BASE PROTONS OF THE DNA AT VARIOUS TEMPERATURES AND MIXING TIMES

Protons	NOE cross-peaks									
	278 K		283 K			289 K		298 K		
	50 ms	100 ms	50 ms	100 ms	180 ms	50 ms	180 ms	50 ms	100 ms	180 ms
T7 Methyl	–	+	––	––	––	––	–	––	–––	–––
T8 Methyl	–	–	––	––	––	––	–	––	––	–
C3, C5H	++	++	+++			+		+	++	+
C11, C5H	++	+	+++			++		+	+++	++
C9, C5H	+	++	++			+		+	++	++
C1, C5H		+++	+++			+	+	++	+	+
A5, C2H	++	++	++	+		+	+	+	+	+
A6, C2H	++	++	+++	++		++	+	+	+	+
G4, G10, C8H	+	++	+++	++	++	+		+	+	+
G12, C8H	+	+	+++	++	++	+	+	+	+	+
G2, C8H	+	++	+++	++	++	+	+	++	+	+
C3, C6H			+		+	+			++	+
C9, C6H	+	+	+		+	+	+++	++	++	+
T8, C6H	+	+	+		+	+	+++	++	++	+

'+' = positive NOE cross-peak; '–' = negative NOE cross-peak; stronger NOEs are indicated by more signs.

sites have different lifetimes, i.e., a distribution of water molecule lifetimes at DNA binding sites. The dynamic range and precision of the relaxation dispersion data do not permit a detailed characterization of such a distribution. Nevertheless, the proton relaxation dispersion data of Fig. 1 require that such a distribution cannot include more than a few irrotationally bound water molecules. Since the X-ray structure provides a rather complete picture of many bound water molecule sites, we conclude that the average lifetime for the water molecules in these sites is short compared with the rotational correlation time for the duplex.

The discussion of the crystal structure identifies 65 water molecules that are associated with the 22 phosphate groups of the backbone (Kopka et al., 1983). Of these, 42 water molecule oxygen atoms are reported to be within 3.5 Å of a phosphate oxygen. The magnetic relaxation dispersion experiment is sensitive to these water molecules as well. Thus, the average lifetime of the water molecules associated with the phosphate groups must also be short compared with the rotational correlation time of the duplex. We may invert the analysis of Eq. 7 and assume that instead of the rotational correlation time, it is the chemical exchange time that limits the bound site correlation time. With the assumption that all 65 water molecules are the same, at 278 K, the average lifetime estimate that results is 1 ns. We point out that in this case, the position of the relaxation dispersion would be domi-

TABLE 4
NOE CROSS-PEAKS BETWEEN WATER PROTONS AND DEOXYRIBOSE PROTONS OF THE DNA AT VARIOUS TEMPERATURES AND MIXING TIMES

Protons	NOE cross-peaks									
	278 K		283 K			289 K		298 K		
	50 ms	100 ms	50 ms	100 ms	180 ms	50 ms	180 ms	50 ms	100 ms	180 ms
C1, C2"H	+	+	+	+	+	+	+	+	+	+
T7,8, C2"H	+++	+++	+++	++	++	++	++	+++	++	+++
A5, G2, C2"H	++++	++++	++++	++++	++++	+	++			
A6, C2"H	+++	+++	++	++	+	+	++		+	
C1, C5'H	+	+	–	+	+++	–	++		––	––
G2, C4'H		+++	+++		+++		+	++	++	+
G10, C4'H	++	++	++						++	
C1, C2'H								+++	+++	+++
T7, C2"H								+++	+++	++
C9, C2'H								++	++	+
T8, C2'H								+	+	+
C3, C2"H								++	+	++

'+' = positive NOE cross-peak; '–' = negative NOE cross-peak; stronger NOEs are indicated by more signs.

nated by a slower process, such as the exchange of the labile protons of the DNA, and the bound water contribution is associated with the higher field tail which is not completely characterized in the present data set. Nevertheless, the consequences of the magnitude of the low-field relaxation rate remain as discussed above.

Overhauser effects

The Overhauser experiments provide a different and higher resolution characterization of the water molecule binding to macromolecules that also provide information about the lifetimes of the association. A difficulty with Overhauser effects is that a coupling between water protons and macromolecule protons may be carried by several mechanisms in addition to direct or through-space dipole–dipole interactions. For example, Wüthrich and co-workers (Liepinsh et al., 1992) pointed out in their earlier detailed study that the chemical shift of the water protons depends strongly on temperature, covering the range from 5.01 ppm at 277 K to 4.45 ppm at 341 K. This range overlaps the region of deoxyribose C3' protons (Hare et al., 1983). Thus, cross-peaks may develop because of indirect coupling through these protons and appear as though the source were water. Based on the structure, protons that are within 4.5 Å of the deoxyribose C3' proton include: C5'H, C4'H, C2'H, C1'H, C3:C6H, T7:C6H, T8:C6H, C11:C6H, C9:C6H, and G12:C8H. As shown in Fig. 2, some of the cross-peaks appear at low temperature, such as C2", and some at high temperature, such as C2' because of the temperature-dependence of the water proton shift.

The spectra of Figs. 2 and 3 are similar to those reported by the Wüthrich group. Although Tables 3 and 4 include several mixing times, we discuss only the 50-ms data, because the longer times may involve complications caused by spin diffusion within the complex. Table 3 shows that there are positive cross-peaks or negative NOEs observed at position 2 of A5 and A6, which was also reported by the Wüthrich group (Liepinsh et al., 1992). Overhauser effects between water and protons of the major groove at position 5 of C1, C3, C9, and C11 and position 8 of G4, G2, and G10 are also discernable. The negative sign of the Overhauser effect implies that the motion of the water be slow compared with the Larmor frequency, i.e., slow compared with approximately 0.3 ns. Because of the relaxation dispersion discussion above, unless the Overhauser effects are carried by three or four water molecules, the water molecule lifetimes, even in the minor groove, must be short compared with 25 ns. Based on these constraints, the water molecules responsible for the nuclear Overhauser effects detected appear to be in the range of 1–25 ns and include sites in both the minor and major grooves of DNA.

Liepinsh et al. (1992) discussed only cross-peaks from the minor groove. Although there are some intensity differences caused by the different pulse sequences used, the conclusions of Liepinsh et al. are well supported by the present data. The higher concentration of DNA used in the present experiments may shift the correlation slightly and make the cross-peak intensity somewhat higher; nevertheless, several cross-peaks between the water and base protons in the major groove are apparent. The crystal structure, which also provides positions for a number of oxygen atoms ascribed to the water molecules, implies that there are approximately 23 water molecules within 3.5 Å of a nitrogen atom. Though the distance choice is arbitrary, this distance is shorter than would normally be implied by only van der Waals contacts. Of these 23 molecules, 16 are in the major groove. If there is a distribution of water molecule lifetimes, it is quite reasonable that a few water molecules may have residence times at groove sites longer than 0.3 ns.

Inspection of Table 3 and Fig. 2 shows that the cross-peak intensity generally decreases with increasing temperature, which is consistent with the lifetime of the water molecules and the effective correlation time decreasing with increasing temperature. On the other hand, direct chemical exchange peak intensities, for example the largest peak on the left of spectrum (B) in Fig. 2, generally increase with increasing temperature. Therefore, the cross-peak intensities that are tabulated and decrease in amplitude with increasing temperature are not caused by chemical exchange of labile protons, which was pointed out by Liepinsh et al. (1992).

In summary, this combination of high-resolution and relaxation dispersion data places additional constraints on the water molecule lifetimes when it is associated with the DNA duplex. There is no evidence from either the relaxation dispersion or the NOE data that water in the major groove is substantially slowed by its interaction with the DNA. The water in the minor and major grooves may experience some distribution of lifetimes; however, the magnitude of the water proton relaxation rate at low field requires that either only a few water molecules are bound for a period on the order of the rotational correlation time of the DNA, or the average lifetime is short compared with approximately 25 ns. With the exception of the methyl protons of thymine, which may be complicated by methyl rotation, the observable nuclear Overhauser effects between water protons and resonances in the minor and major grooves are negative; therefore, the lifetimes for these water molecules must exceed the reciprocal of the Larmor frequency, or approximately 1 ns.

Acknowledgements

We would like to thank Dr. Bayard K. Fetler for helpful discussions on the water-suppression pulse sequence. This work was supported by a grant from the National Institutes of Health (GM-34541) to R.G.B.

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