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A 300 MHz and 600 MHz proton NMR study of a 12 base pair restriction fragment: investigation of structure by relaxation measurements

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ABSTRACT

The ^1H NMR spectrum of a 12 base pair DNA restriction fragment has been measured at 300 and 600 MHz and resonances from over 70 protons are individually resolved. Relaxation rate measurements have been carried out at 300 MHz and compared with the theoretical predictions obtained using an isotropic rigid rotor model with coordinates derived from a Dreiding model of DNA. The model gives results that are in excellent agreement with experiment for most protons when a 7 nsec rotational correlation time is used, although agreement is improved for certain base protons by using a shorter correlation time for the sugar group, or by increasing the sugar-base interproton distances. A comparison of non-selective and selective spin-lattice relaxation rates for carbon bound protons indicates that there is extensive spin diffusion even in this short DNA fragment. Examination of the spin-spin relaxation rates for the same type of proton on different base pairs reveals little sequence effect on conformation.

INTRODUCTION

The determination of the precise conformation of DNA and RNA in solution has been a goal of many spectroscopic studies. Of the many solution state techniques that have been used (infrared[1], Raman[2-4], circular dichroism[5-7], low angle x-ray diffraction[8] and other optical methods[9,10]), NMR is the one spectroscopic tool that can, in principle, provide enough data to determine the complete solution state structure of DNA. Until now this method has only been used to probe limited aspects of the helical structure[11-16]. We believe that through the use of small DNA helical fragments and by application of the more sophisticated NMR relaxation techniques using the highest field spectrometers available it may now be possible to reach this long sought goal. The reason for focusing on relaxation measurements derives from the fact that the spin-lattice relaxation rates ($R_1 = 1/T_1$) and the spin-spin relaxation rates ($R_2 = 1/T_2$) vary with the inverse sixth power of interproton distances[17]. Internal molecular motion can be important in the relaxation

process, but by choosing a small molecule where the overall motion of the molecule is sufficiently fast the effect of the internal motions is diminished. Furthermore, it is often possible to separately evaluate the different contributions to the relaxation process and in this way independently determine interproton distances and correlation times characterizing internal motion.

Preliminary studies of this type conducted on sized DNA with random sequences have already been reported[18]. In the present work we present the first high field (600 MHz) NMR spectra and results of relaxation measurements at 300 MHz on a 12 base pair DNA restriction fragment with the sequence d(CCGCACTGATGG)•d(CCATCAGTGCGG). These relaxation measurements have permitted us to evaluate the various factors which are involved in the proton relaxation mechanisms and lay the groundwork for a much more intensive study of the relaxation behavior of individual resonances from which it should be possible to determine many important interproton distances in the DNA molecule, and hence its structure in solution.

METHODS AND MATERIALS

Instrumentation. The 300 MHz proton NMR spectra were obtained with a home-built FT spectrometer which utilized a Varian HR 300 Magnet and cross-coil probe[18]. For samples in D_2O the spectra and relaxation measurements were obtained using standard FT techniques. With samples in H_2O the dynamic range problem arising from the intense solvent peak was overcome using the long pulse FT method as described in detail elsewhere[18]. In this method a weak RF pulse is applied to resonances of interest and the power level and pulse length are adjusted to minimize the H_2O peak. In the present experiments the RF pulse was located about 2500 Hz from the water peak, and in this case a 90° pulse of about 360 μsec and 151 milligauss was used. The residual water signal was further attenuated with a Rockland model 442 low pass filter in the audio stage of the receiver. Quadrature phase detection was used with a sweep width of ± 2500 Hz to prevent the residual water signal from folding into the lowfield spectral region. For the T_1 and T_2 experiments 1000 FIDs were accumulated with a Nicolet 1180 computer. In most cases 80 to 90% inversions were obtained for the T_1 inversion-recovery experiments. The 600 MHz spectra were obtained at the Regional Instrument Center at Carnegie-Mellon Institute.

Samples. The preparation of the 12 base pair Hae III restriction fragment from pVH51 is described in detail elsewhere (W. Hillen, R.D. Klein

and R.D. Wells, manuscript in preparation). For the NMR measurements 1.5 mg of this material was dissolved in 0.12 ml of solution containing 350 mM NaCl, 85 mM phosphate at pH = 7.3.

RESULTS

Spectral Assignments. The 600 MHz proton NMR spectrum of the 12 base pair fragment is shown in Fig. 1. It is remarkably well resolved and spin-spin splittings are evident on a number of resonances. From a consideration of chemical shifts, coupling constants and relaxation behavior most of the resolved resonances in the spectrum can be assigned to specific types of protons in the molecule.

Resonances from the imino protons of bases in Watson-Crick base pairs occur in the lowfield region (15-10 ppm)[12-16,19] and in the 600 MHz spectrum only 10 of the expected 12 resonances are observed at 25°C (Fig. 1A). The resonances from the two terminal G•C base pairs are absent at this temperature, but 300 MHz experiments conducted at lower temperatures show the full complement of twelve resonances at 1°C. (Early, Kearns, Hillen and Wells, unpublished results). Early melting of resonances from terminal base pairs is usually observed in the spectra of short oligonucleotide fragments due to fraying at the ends of the helix[13,20]. In fact, the early melting behavior can be used to assign resonances at 13.25 and 12.95 ppm to base pairs 1,2,11 and 12 and a careful analysis of the temperature dependence of the lowfield spectrum should permit other resonances to be assigned. The four lowest field resonances can be assigned to the four A•T base pairs in the molecule since they are all located below the intrinsic lowfield position expected for a G•C base pair (13.6 ppm)[19].

In D_2O the only resonances which occur in the 9-7 ppm region are due to base protons [A(H-2,8), G(H-8), C(H-6), T(H-6)] and integration of the spectrum shown in Fig. 1B shows that all 28 of the expected resonances are present. The four lowest field resonances in Fig. 1B are assigned to A(H-8) protons since they have the lowest field intrinsic positions (8.4 ppm)[11,12,14] and are expected to receive only a small ring current shift from neighboring bases. The four resonances from A(H-2) are easily identified in the partially relaxed T_1 and T_2 experiments (vide infra) because they are the most slowly relaxing protons. Resonances from C(H-6) protons can be identified by their large (7.1 ± 0.2 Hz) scalar coupling with the C(H-5) protons on the same base. The majority of the G(H-8) resonances are located between 8.0-7.8 ppm whereas

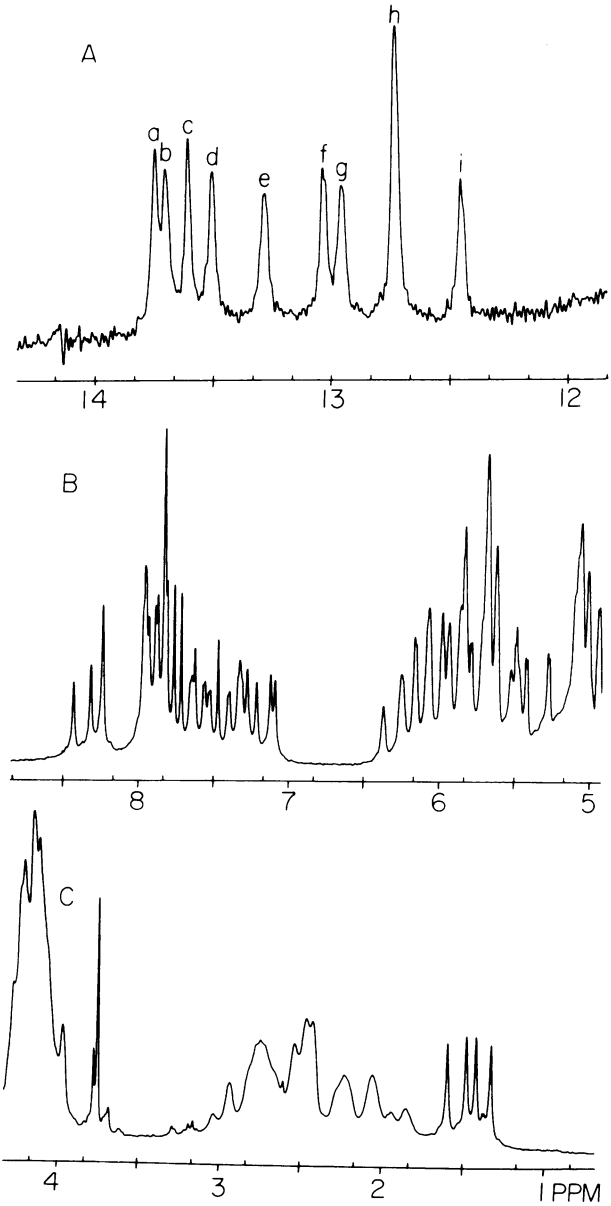

Figure 1. The 600 MHz correlation spectra of the 12 base pair DNA restriction fragment. (A) shows the lowfield spectrum taken in H_2O, (400 scans), (B) and (C) show the non-exchangeable proton spectrum in D_2O (800 scans). The probe temperature was 20°C. A 0.2 Hz line broadening was used to enhance the signal-to-noise in all spectra.

resonances from T(H-6) are expected between 7.5-7.0 pm. Selective T_1 experiments discussed below support the general assignments of the T(H-6) and G(H-8) resonances, since selective T_1^s values for T(H-6) are expected to be shorter than for G(H-8).

Resonances from C(H-5) and ribose H-1' protons overlap with each other in the 6.5-5.0 ppm region, but most of the C(H-5) resonances can be identified in a resolution enhanced spectrum (not shown) as they are all split by about 7.0 Hz through coupling with the C(H-6) protons. In preliminary decoupling experiments we have unambiguously identified 4 of the C(H-5) resonances and at the same time correlated C(H-5) and C(H-6) resonances from the same base. The resonances from sugar protons exhibit a more complicated splitting pattern due to spin-spin coupling with other sugar protons.

Resonances from the sugar H-3' protons are at ~4.8 ppm and can only be observed in a partially relaxed T_1 inversion-recovery experiment because of interference from the large HOD peak. Resonances from sugar H-5',5'' and H-4' protons appear in the large peak centered around 4.3 ppm and are only partially resolved from one another. The sugar H-2',2'' resonances are located between 3.0 to 1.9 ppm and are poorly resolved. The four resonances from the methyl protons of T are easily assigned to the four peaks centered about 1.5 ppm.

In total, well over 70 individual resonances are observed in the region between 15-0 ppm, and most can be assigned to specific kinds of protons. If ring current shift calculations are used, tentative assignments of many resonances to specific base pairs in the helix can be made; however, definitive assignments require more information such as would be provided by 2D-FT experiments and measurements of the temperature dependence of the spectra.

Relaxation Measurements. Extensive relaxation measurements have been conducted at 300 MHz and some of these results are presented in Figs. 2, 3 and 4. Three different types of relaxation measurements were conducted. In measuring the spin-spin relaxation rates (R_2) the standard spin-echo pulse sequence ($90°-\tau-180°-\tau$) was used. Selective spin-lattice relaxation rates (R_1^s) were measured for both the lowfield resonances and for the aromatic resonances by the inversion-recovery method ($180°-\tau-90°$) in which pulse widths and power were adjusted to more or less selectively excite only one group of protons (i.e., aromatic, imino). In measuring the non-selective spin-lattice relaxation rates (R_1) all C-H protons were simultaneously inverted.

The long pulse method[18] was used to measure selective R_1^S and R_2 rates for the lowfield resonances and these results are shown in Fig. 2 and in Table 1. Although we refer to these as selective experiments, in fact they are only semi-selective as all imino protons are simultaneously inverted, thus

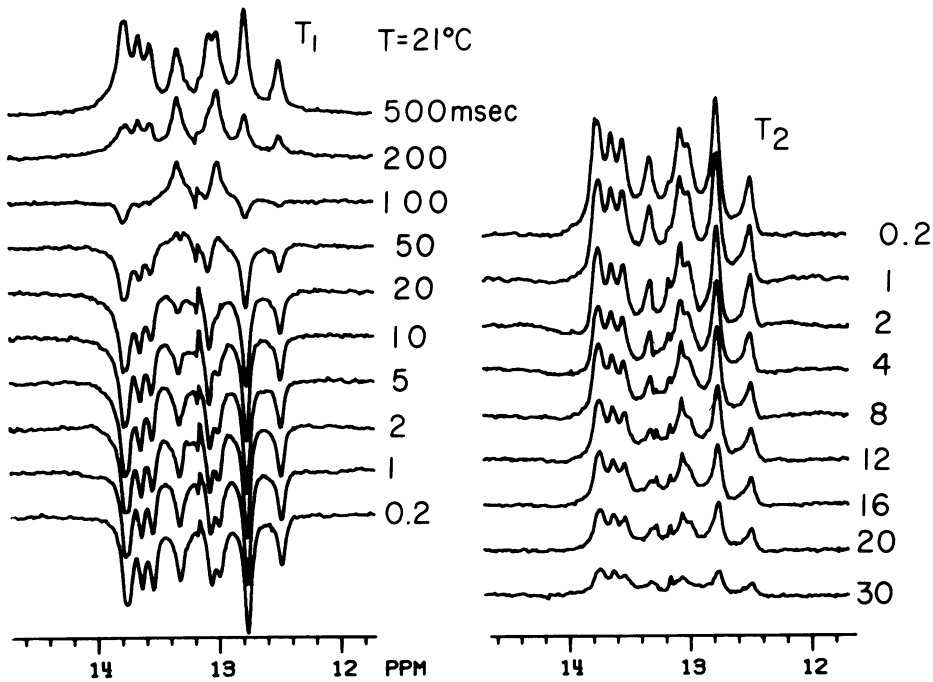

Figure 2. Relaxation measurements on the lowfield resonances in H_2O. In the semi-selective R_1^S (T_1^S) experiment shown in (a) the sample was subject to the (180°-τ-90°) inversion recovery pulse sequence, while the R_2 (T_2) experiment shown in (b) used the Hahn spin echo pulse sequence (90°-τ-180°-τ). In both experiments the 180° and the 90° pulse lengths were 781.5 and 390.7 μsec respectively. The delays, τ, are indicated in milliseconds to the right of each spectrum. A 2.00 second recovery time was used in accumulating 1000 FIDs by quadrature phase detection. The RF pulse was located at about 13.15 ppm and the sweep width was ±2500 to prevent the residual water signal from folding into the lowfield region. The probe temperature was maintained at 18.0 ± 0.5°C with a Varian temperature controller, and was checked before and after the experiment. Spectra were baseline corrected using a quadratic equation fit to the baseline on both sides of the lowfield resonances. R_1 and R_2 relaxation rates were calculated using the Nicolet 1180 software and are given in Table 1. A three parameter fit was used in the inversion recovery experiments, and the resulting inversion parameter (w) varied from 0.98 to 0.81 for the various peaks a-i.

Table 1. A summary of spin-lattice (R_1) and spin-spin (R_2) relaxation rates observed for the lowfield resonances of a 12 base pair DNA restriction fragment at 21°C

Relaxation Rate	A•T Resonances(i)				G•C Resonances(i)				
	a	b	c	d	e	f	g	h	i
R_1, sec^{-1}	4.2	4.2	5.5	6.0	17.8	7.2	13.0	5.5	5.1
$\langle R_1 \rangle$(ii)		5.0				6.0(iii)			
R_1^{mag}(iv)		1.7				4.5			
R_2, sec^{-1}	25	22	21	25	34	27	30	25	26
$\langle R_2 \rangle$(ii)		22					27		
R_2^{mag}(iv)		20					26		

(i) Spectrum shown in Fig. 1.

(ii) Average value.

(iii) Average calculated neglecting peaks e and g.

(iv) Value obtained after subtracting out exchange contribution.

rendering the effects of spin exchange between neighboring imino protons on R_1 negligible[17].

With D_2O samples the standard inversion-recovery and spin echo techniques were used to measure the spin-lattice and spin-spin relaxation rates (R_1 and R_2) on the non-exchangeable C-H protons. In the non-selective experiments (not shown) where all C-H protons are simultaneously inverted we find that all resonances relax with a common spin-lattice relaxation rate of 0.6 sec^{-1}, except for the A(H-2) protons where $R_1 = 0.4$ sec^{-1}. By contrast, measurement of spin-spin relaxation rates, R_2, (shown in Fig. 3 and summarized in Table 2) yielded values ranging from 70 to <10 sec^{-1}. This observation, and the fact that R_2 is 100 times larger than R_1 for some resonances suggests that the anomalously small non-selective R_1 rates are due to spin diffusion[21]. This is confirmed by the semi-selective relaxation experiment shown in Fig. 4 in which only resonances in the 8.5-7.0 ppm region are selectively inverted and allowed to relax. In all cases R_1^s rates are considerably larger than those

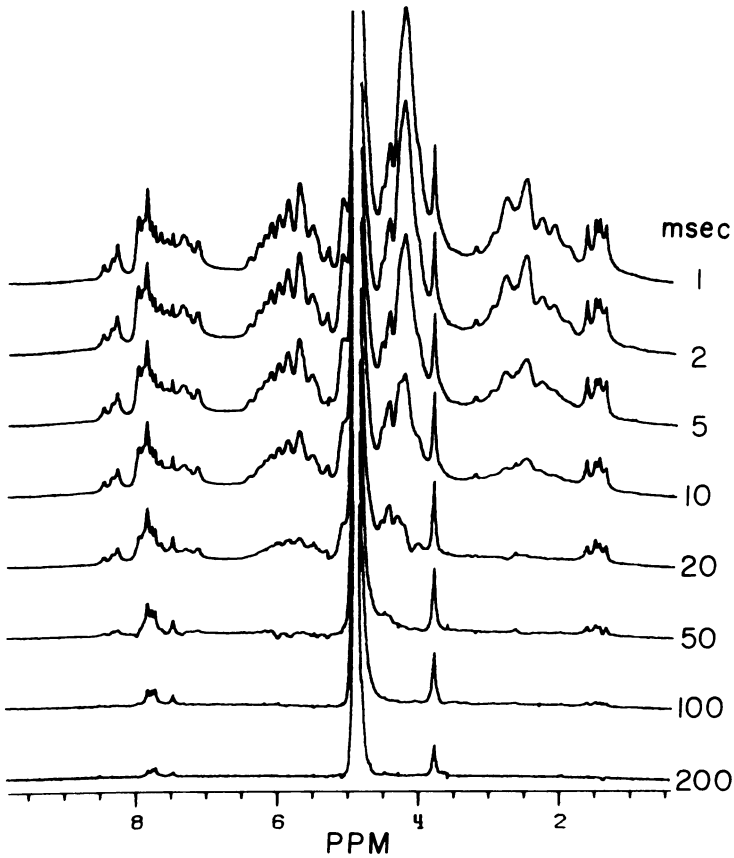

Figure 3. R_2 rate measurement on non-exchangeable protons in the 12 base pair fragment at 22°C in D_2O. The Hahn spin echo method ($90°-\tau-180°-\tau$) was used and τ delays are indicated to the right of each spectrum. An 8 second recovery time was used in this experiment and 800 FIDs were accumulated using quadrature phase detection. The peak at 3.75 ppm is due to the buffer.

measured in the non-selective measurement. Thus, depending upon the type of relaxation experiment conducted (non-selective or semi-selective) quite different spin-lattice relaxation rates are obtained. These results conclusively demonstrate that there is extensive spin diffusion even in a molecule of this size, and consequently measurements of non-selective spin-lattice relaxation rates for C-H protons are relatively uninformative with

Table 2. A Comparison of Proton Spin-Spin (R_2) and Spin-Lattice (R_1) Relaxation Rates Measured for a 12 Base Pair Restriction Fragment at 21°C with Rates Computed Using an Isotropic Rotor Model

Proton of Interest	Neighboring Protons Type	Distance (Å)	Observed Relaxation Rates, sec^{-1}	Calculated[i] Relaxation Rates, sec^{-1}	Ratio Calc./Obs.
Thymine	A(NH_2)	2.6	R_1=1.7	1.5	0.88
N-H					
	A(H-2)	3.0	R_2=20	21	1.05
Guanine	G(NH_2)	2.4	R_1=4.5	3.0	0.66
N-H					
	C(NH_2)	2.6	R_2=26	25	0.97
Purine	Ribose				
H-8	1'	2.5	R_1=6	R_1=12	2.0
	2'	2.2			
	2''	2.0	R_2=20±2	R_2=33	16±2
	5'	2.5			
C(H-6)	C(H-5)	2.4			
	Ribose				
	1'	2.5	R_1=7.2	R_1=14	2.0
	2'	2.2	R_2=35	R_2=40	1.15
	2''	2.0			
	5'	2.5			
C(H-5)	C(H-6)	2.4			
	Ribose				
	1'	2.5	R_1=--	R_1=14	
	2'	2.2	R_2=33	R_2=40	1.2
	2''	2.0			
	5'	2.5			
Ribose	Pu(H-8) or Py (H-6)	2.5			
(H-1')	C(H-5)	2.5	R_1=--	R_1=10.5	
	Ribose				
	5'	2.0	R_2=33	R_2=29	0.90
	2'	3.0			
	2''	2.4			
Ribose	Ribose				
2' (or 2'')	2'' (or 2')	1.8	R_1=--	R_1=18(25)	
	1'	2.4 or 3.0		R_1[ii]=9(14)	
	5'	3.0	R_2=62	R_2=53(70)	0.86(1.1)
	Pu(H-8)				
	or	2.0			
	Py(H-6)				
	C(H-5)	2.0			
Adenine					
(H-2)	5 Protons	~5	R_1=<0.5	R_1=0.14	>0.4
	2 Nitrogens	2	R_2=<10	R_2=0.56	>.13

(i) τ_c = 7 nsec. All R_1 calculations are selective rates unless otherwise specified.

(ii) Biselective-excitation of both 2' and 2''.

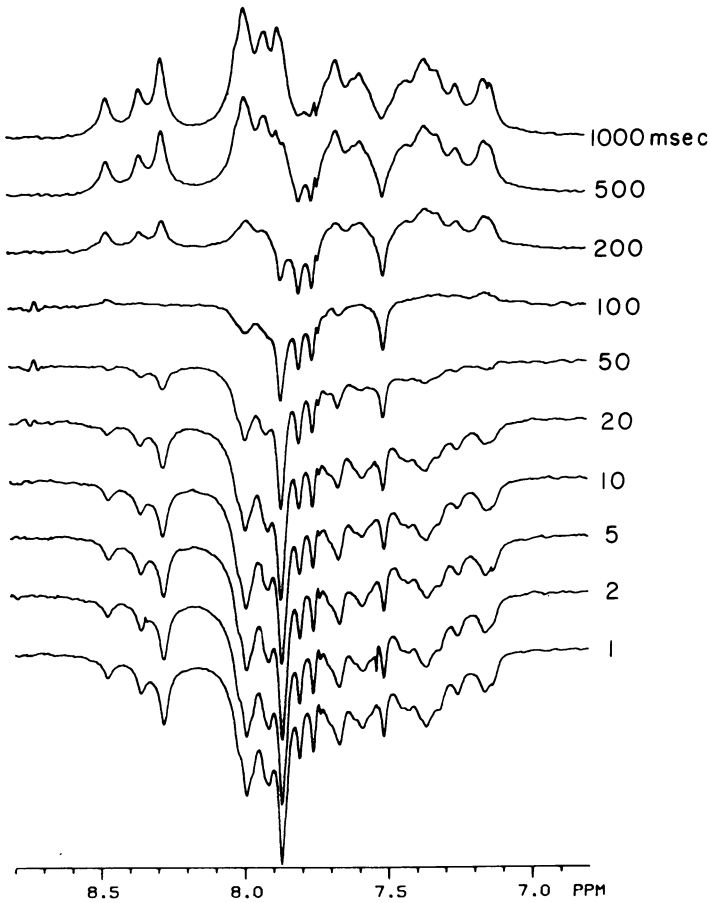

Figure 4. Measurements of semi-selective R_1^S (T_1^S) of the aromatic resonance at 22°C in H_2O. The inversion-recovery method was used with the 180° and the 90° pulse width of 1250 and 675 μsec respectively. The RF pulse was located at 7.75 ppm while 1000 pulses were accumulated. Delay times, τ , are indicated to the right of each spectrum in milliseconds. Quadrature phase detection was used with a sweep width of ±1000 Hz to prevent the residual HOD signal from distorting the aromatic spectrum.

regard to the relaxation behavior of individual protons. However, this information can be obtained by carrying out selective relaxation measurements and the results of these measurements are analyzed in the next section.

DISCUSSION

A major goal of this reseach is to use relaxation techniques to obtain precise information about the conformation of DNA and the nature of its conformational fluctuations. Both factors may play an important role in the recognition of specific DNA sequences by proteins, and certainly conformational fluctuations are important to the flexibility of DNA[22-25], its interaction with proteins and the way in which it is packaged in cells[26-30]. To the extent that both conformational and dynamic properties can be accurately monitored by relaxation methods, this approach may be used to study the effect of various environmental factors (ionic strength, pH, metal ions, polyamines, proteins, temperature) on the properties of DNA. Relaxation measurements may at last provide us with the tool required to study in detail the precise structure of DNA in solution. We now give a brief theoretical analysis of the relaxation measurements and discuss the information that can be derived from those experiments.

Relaxation Theory. If we assume that the magnetic contributions to the relaxation rates are entirely due to the dipole-dipole interactions, the expressions derived by Abragam[17] for the dipolar relaxation of one spin I by a second spin S can be used to calculate relaxation rates. For a selective spin relaxation measurement in which a 180° pulse is applied to just the I spins, the initial rate for the relaxation of I is just $R_1^S = 1/T_1$ where

$$R_1^S = \frac{2\gamma_I^2\gamma_S^2\hbar^2 S(S+1)}{3r^6}\{J_0(\omega_I-\omega_S) + 3J_1(\omega_I) + 6J_2(\omega_I+\omega_S)\} \qquad (1)$$

and r is the internuclear distance, γ_I and γ_S are the magnetogyric ratios for the I and S spins respectively, $J_n(\omega)$ is the spectral density at frequency ω resulting from appropriate molecular motions which modulate the I-S dipolar interaction.

The corresponding equation for the dipolar contributions to the spin-spin relaxation rate is

$$R_2^{I-S} = \frac{\gamma_I^2\gamma_S^2\hbar^2 S(S+1)}{3r^6}\{4J_0(0) + J_0(\omega_I-\omega_S) + 3J_1(\omega_I) + 6J_1(\omega_S) + 6J_2(\omega_I+\omega_S)\} \qquad (2)$$

If more than one spin contributes to the relaxation of the I spin, the total relaxation rate will be the sum of contributions from each spin

(assuming no cross-correlation of their motion). To apply these expressions to the 12 base pair fragment we make the following additional assumptions.

(i) Overall molecular motions dominate internal motions in the relaxation process.

(ii) A single rotational correlation time, 7 nsec at 21°C, is used to characterize the overall molecular motion. Use of a single relaxation time is justified by the fact that the rotational correlation times for axial spinning and end-over-end tumbling calculated by the Perrin[31] formulae differ from each other by only 25%. Since the viscosity of the solution is unknown, the value of 7 nsec was chosen empirically to give the best fit of the experimental data.

(iii) Relaxation mechanisms involving chemical shift anisotropy, scalar interactions, chemical exchange, etc., are assumed negligible.

(iv) DNA coordinates derived from a Dreiding model for B-form DNA with 10 base pairs per turn were used to derive interproton distances (see Table 2).

For an isotropic rotor, the spectral densities are

$$J_n(\omega) = \frac{\tau_c}{5(1 + \omega^2\tau_c^2)} \qquad (3)$$

and in the slow motion limit where $\omega\tau_c \gg 1$ we predict $J_0(0) > J_1(\omega) > J_2(2\omega)$. Consequently for proton NMR at 300 MHz where $\omega = 1.8 \times 10^9$ rad/sec, proton-proton dipolar contributions to the relaxation rates are dominated by the $J_0 = \frac{\tau_c}{5}$ term. The above expressions can be used along with the DNA coordinates derived from the model to compute the spin-lattice relaxation rates ($R_1^S = 1/T_1^S$) for selective inversion of a specific group of resonances (eg, the lowfield resonances, the aromatic protons, etc.) and the spin-spin relaxation rates ($R_2 = 1/T_2$) arising from neighbor proton or nitrogen dipolar interactions. These results are shown in Table 2 where they are compared with the appropriate experimental results obtained at 21°C.

Comparison of Theory and Experiment

(i) Lowfield Relaxation Behavior: We first consider the relaxation data for the lowfield resonances presented in Table 2. Neglecting for the moment the R_1^S and R_2 values obtained for resonances e and g, we find excellent agreement between experiment and theory using a rotational correlation time of

7 nsec. Various internal comparisons are possible and each provides checks on different assumptions which go into the theory. For example, the prediction that $R_1^S(G\cdot C)/R_1^S(A\cdot T) = 2.0$ (Table 2) for the lowfield resonances follows from the assumed geometry of the base pair and the assumption that the J_0 terms in Eq. (1) dominate, but it does not depend upon knowledge of the magnitude of J_0 or the rotational correlation time. Prediction of the absolute magnitudes for R_1^S, on the other hand, does depend on these latter quantities, and using a rotational correlation time of 7 nsec (corresponding to a viscosity of ~1.35 cp) we find that the simple rigid isotropic model gives a good account of most of the lowfield relaxation data at 21°C data. In particular, the absolute values of both R_1^S and R_2 are correctly predicted for the majority of the lowfield A•T and G•C resonances, and the predicted ratios of the rate constants, which are less sensitive to the specific choice of the rotational correlation time, are well accounted for. When there are discrepancies, as for peaks g and e, it is clear that exchange with the solvent is responsible for the disagreement, since these particular resonances arise from base pairs at or near the termini of the helix and hence are very susceptible to exchange with the solvent[20].

The fact that the relaxation behavior of the lowfield resonances is well accounted for by the theory supports the validity of the assumptions regarding the base pair geometry and the use of a single rotational correlation time. It also indicates that in so far as the relaxation of the lowfield resonances in this short DNA fragment is concerned, internal motions (bending, propellering, twisting of the base pairs relative to the molecular axes) are unimportant compared with the overall motions of the molecule in the 7 nsec range. This agrees with our earlier work[32] in which we concluded from studies of higher molecular weight DNA that large amplitude internal motions involving reorientation of the planes of the base pair are relatively slow (between 10-100 nsec). It does not agree, however, with the recent suggestion of Hogan and Jardetzky[33] that the planes of the bases undergo large amplitude (±20°) internal motions with a 1 nsec time constant. We note that their measurements were carried out using partially fractionated random sequence DNA and some of their relaxation measurements were obviously complicated by spin diffusion. We now examine the relaxation behavior of the resonances from non-exchangeable protons.

(ii) Non-Selective R_1 Measurement on C-H Protons and Evidence for

Spin Diffusion: In a non-selective T_1 measurement where all proton spins in the molecule are simultaneously inverted, we find that, except for the A(H-2) protons which have a relaxation rate of about 0.4 sec^{-1}, all resonances have relaxation rates of about 0.6 sec^{-1}, despite the diversity of interproton distances involved (ranging from 1.8 Å to over 5 Å). This observation is to be contrasted with the R_2 measurements which show values ranging from 62 sec^{-1} to <10 sec^{-1}. Finally, some non-selective R_1 values are 10-100 times smaller than the corresponding R_2 values. While other factors might be contributing, spin diffusion is clearly the principal factor responsible for this "unusual" behavior, and this is confirmed by the semi-selective relaxation experiment shown in Fig. 4. In this experiment, where only the aromatic protons are inverted, we find a considerable range of R_1^S values and most are at least a factor of 10 greater than 0.6 sec^{-1}. Thus, even though the 12 base pair fragment is relatively small by DNA standards, spin diffusion effects dominate the non-selective R_1 measurements. In studies of higher molecular weight DNA, we find non-selective R_1 values are virtually independent of length, with $R_1 \approx$ 0.75 sec^{-1} obtained for 43 to 301 base pair DNA. This insensitivity to DNA length clearly indicates that internal molecular motions are involved in the relaxation process. Finally, the fact that the non-selective R_1 values vary approximately with the square of the applied magnetic field indicates that single quantum relaxation processes are involved. We conclude that any attempt to interpret non-selective R_1 data without considering the effects of spin diffusion are not valid, even for short DNA. It is for this reason that one must focus on selective or semi-selective spin-lattice relaxation measurements to determine structural parameters.

(iii) Analysis of the Selective R_1^S and R_2 Data for C-H Protons: In applying the relaxation theory to the non-exchangeable protons, two complications arise. First, for any given type of proton, a number of interproton interactions generally contribute to the relaxation process, but accurate interproton distances are not known because the solution state structure of a regular DNA helix has not been determined. To obtain the necessary coordinates we used a Dreiding model of DNA with 10 base pairs per turn and a 2' endo sugar pucker. Even with these constraints, some of the required interproton distances were quite sensitive to the precise sugar and backbone conformation used. For example, the separation between base protons

A(H-8), G(H-8) and one of the 2' sugar protons could vary from 2.0 to 2.5 Å, while the corresponding distance to the 2'' sugar proton changes from 2.5 to 2.0 Å. In our calculations we use values of 2.0 and 2.5 Å. The interaction of the sugar protons with the H-5' proton of a neighboring sugar is quite sensitive to the backbone conformation and the value of 2.0 Å used in the calculations may be too small. In addition to these uncertainties, conformational fluctuations will modulate the interproton distances and hence alter the form of the spectral density functions. We, therefore, should not be surprised if the agreement between experiment and theory is not as good as that obtained with the lowfield resonances. The results of the calculations are summarized in Table 2 where they are compared with the appropriate experimental data.

In general the R_2 values are well predicted using a 7 nsec rotational correlation time, although the agreement between theory and experiment would be improved for the purine H-8 and the pyrimidine H-6 protons either by reducing the rotational correlation time associated with base-sugar proton interactions, or by slightly increasing the interproton separation. According to theory, high frequency (1-3 nsec) ±20° fluctuation in the conformation of the sugar would reduce the effective rotational correlation time from 7 to 4.4 nsec[34]. In this regard we note that Bolton and James[35], on the basis of ^{13}C relaxation measurements, have proposed that the correlation times for fluctuations in the sugar backbone conformation are on the order of 6 nsec although they find a shorter time (2 nsec) for the 2' sugar carbon. Hogan and Jardetzky[33] suggest that the correlation time for internal motion in the sugar groups is even shorter (1 nsec). In order to more thoroughly investigate the role of conformational fluctuations of the sugar group using proton NMR it would be better to study higher molecular weight DNA where the rotational correlation times are much larger and hence the relaxation rates are more sensitive to the higher frequency motions.

Finally, we note that the observed relaxation rates for the A(H-2) protons are much larger than those predicted. Part of the discrepancy may be due to the relatively short delay times used in the relaxation measurements and to the fact that experimental artifacts are more important when the relaxation times are long. Further, we note that the A(H-2) proton is quite distant from all other protons in the molecule (5 Å or more) and consequently small changes in the interproton distance of its five neighbor protons would have little affect on R_1^S or R_2 relaxation.

(iv) Sequence Effects on Relaxation Rates: Since relaxation rates vary with the inverse sixth power of the distance between interacting nuclei, they provide a very sensitive method for detecting subtle sequence effects on th local helix conformation. By comparing the relaxation behavior of identical protons located in different base pairs we should be able to detect any differences, if they exist. For example, the four lowest field resonances from A(H-8) are primarily relaxed via interactions with sugar protons, and all have about the same R_1^s and R_2 values and these appear to be very close to those measured for G(H-8). This indicates that both the G(H-8) and the A(H-8) protons experience nearly identical interactions with neighboring sugar protons. Similarly, the four methyl protons of thymine have virtually identical relaxation rates. Slight differences in the R_2 values for the four A(H-2) resonances are noted in Fig. 3 but they are not sufficiently large to infer any significant differences in local conformation. When relaxation measurements are carried out at higher magnetic fields it will be possible to carefully examine the relaxation behavior of each individual resonance and more critically test for sequence effects on conformation. At this point, however, there is little evidence for pronounced sequence effects on local helical conformation in the 12 base pair fragment.

SUMMARY

The relaxation measurements which we have presented here permit a number of interesting conclusions to be drawn about certain aspects of DNA structure, the importance of conformational fluctuations, and both the limitations and potentialities of relaxation measurements on DNA. The fact that the low field proton relaxation data can be well accounted for by the rigid isotropic rotor theory demonstrates that we have a good theoretical basis for understanding proton relaxation phenomenon in DNA. Since the important internuclear dipolar interactions are rather accurately fixed by the hydrogen bonding geometry, the relaxation data on the imino protons do not provide new structural data; however, the fact that 7 nsec rotational correlation time leads to good agreement between theory and experiment indicates that bending and torsional motions in this short DNA fragment are relatively unimportant compared with the overall molecular tumbling. The fact that the relaxation behavior of most of the C-H protons in the molecule can also be well accounted for using a 7 nsec correlation time, suggests that structural parameters derived from a Dreiding model are reasonably accurate. We point out, however, that since at least 4 neighboring protons contribute to

the relaxation of any particular C-H proton, compensating changes in some of the interproton distances would give the same result. By carrying out transient nuclear Overhauser effect measurements, the individual interproton distances could be separately determined. Although the C-H proton relaxation data provide no evidence for major fluctuations in conformation, we note that a slight shortening of the correlation time associated with the sugar-base interactions would improve the agreement between theory and experiment. This is consistent with our earlier linewidth measurements on high molecular weight DNA where we concluded that there was more flexibility in the backbone than in the stacked bases[32]. More recent ^{13}C and ^{31}P NMR studies have provided convincing evidence for conformational fluctuations in the backbone with correlation times on the order of 6 nsec[35]. Because proton spin diffusion is relatively rapid even in this short DNA fragment, the interpretation of non-selective spin-lattice relaxation measurements in which all proton spins are simultaneously inverted is not straightforward. Fortunately, problems arising from spin diffusion can be overcome through the use of selective relaxation measurements. Although there are no significant differences in the spin-lattice relaxation rates observed for the methyl resonances or the A(H-8) resonances of the A•T base pairs, slight differences are noted for the A(H-2) resonances. This indicates that the local conformation of the DNA is virtually identical for the four A•T base pairs. In future studies we shall report on the use of various relaxation measurements to investigate other DNA restriction fragments covering a range of lengths.

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