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NMR Distance Measurements in DNA Duplexes: Sugars and Bases Have the Same Correlation Times†

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ABSTRACT: To evaluate whether the sugar moieties of short DNA duplexes exhibit local motion of sufficient amplitude to affect interproton distance measurements, we have carried out a series of time-dependent NOESY experiments at increasingly shorter mixing times on dodecamer DNA duplexes. By use of the cytosine H5–H6 vector as a known distance in the bases and the geminal 2′H–2″H vector as a known distance in the sugars, the corresponding apparent cross-relaxation rates were sampled at various mixing times. While the ratio of the inverse sixth power of these two fixed distances is in the range 6–7, when the system is sampled at 100 ms the apparent initial rate of growth of the 2′H–2″H NOESY crosspeak is only 1.9–2.0 times faster than that of the H5–H6 crosspeak—in agreement with the results of Clore and Gronenborn [Clore, G. M., & Gronenborn, A. M. (1984) *FEBS Lett. 172*, 219; (1984) *FEBS Lett. 175*, 117] and of Gronenborn and Clore [Gronenborn, A. M., & Clore, G. M. (1985) *Prog. NMR Spectrosc. 17*, 1]. This observation was interpreted to indicate the existence of internal mobility with a 3-fold shorter correlation time for the sugar moieties in DNA and led to the use of this shorter correlation time to estimate sugar–sugar proton distances and many sugar–base proton distances in subsequent DNA structure determination. We have examined 2′H–2″H cross-relaxation and H5–H6 cross-relaxation at 100, 90, 60, 30, and 15 ms in dodecamer DNA duplexes. The ratio of the rates increases smoothly from 1.9–2.0 to 6–7 as the mixing time is reduced. The results show that the scaling discrepancy at 100 ms is not due to internal motion in the sugar but is simply due to spin diffusion from the rapidly relaxing 2′H,2″H geminal protons. The lack of significant large-amplitude nanosecond local motion in the sugars casts doubt on the accuracy of interproton distances measured on the assumption of a 3-fold shorter correlation time for sugar protons and hence on the validity of DNA structures generated from such distances.

The development of high-field NMR[1] spectrometers operating in the 500-MHz range has led to the ability to determine the structures of small proteins (Havel & Wüthrich, 1985; Williamson et al., 1986) and short DNA duplexes (Hare & Reid, 1986; Nilsson et al., 1986; Hare et al., 1986a,b; Nilges et al., 1987a,b) directly in solution. Common to all these studies is the measurement of distances between assigned protons of known residues within the polymer [for reviews, see Wemmer and Reid (1985), Gronenborn and Clore (1985), and Reid (1987)]. In the case of DNA structure determination, these interproton distances can be used as constraints, either to refine an idealized model double helix (Nilsson et al., 1986; Nilges et al., 1987a,b; Clore et al., 1985) or to calculate a structure de novo by using distance geometry methods (Hare & Reid, 1986; Hare et al., 1986a,b).

The interproton distances to be used in calculating a DNA structure or in refining a model duplex by restrained least squares (Clore et al., 1985) or by restrained molecular dynamics (Nilsson et al., 1986; Nilges et al., 1987a,b) are experimentally obtained from time-dependent NOE measurements using either one-dimensional (Nilsson et al., 1986; Nilges et al., 1987a) or two-dimensional (Hare & Reid, 1986; Nilges et al., 1987b) NMR data. The determination of interproton distances from NOE buildup rates involves both approximations and assumptions, either of which can jeopardize the accuracy of the distance measurement and hence the correctness of the resulting structure.

The approximation used in NOE distance measurement is the two-spin approximation in which only the rate of dipolar magnetization transfer between proximal spins i and j is monitored, and all other spins are ignored. For a two-spin system, the rate of magnetization transfer is (Solomon, 1955)

$$R_{1(ij)} = \frac{\gamma^4\hbar^2}{10r_{ij}^6}\left[\frac{6\tau_c}{1+4\omega^2\tau_c^2} - \tau_c\right] = -\frac{\gamma^4\hbar^2\tau_c}{10r_{ij}^6} \quad \text{when } \omega\tau_c \gg 1 \quad (1)$$

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[1] Abbreviations: NOE, nuclear Overhauser effect; NOESY, nuclear Overhauser effect spectroscopy; NMR, nuclear magnetic resonance; DNA, deoxyribonucleic acid.

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Thus, the $i - j$ magnetization transfer rate depends on the distance r_{ij} and the correlation time, τ_c, of the ij vector. For 10–15-bp DNA duplexes at ca. 30 °C, the global τ_c of the cylindrical double helix is ca. 4–5 ns, and at 500 MHz, $4\omega^2\tau_c^2 \approx 1000$, rendering the first term in parentheses negligible if the ij vector has the global correlation time (i.e., assuming no internal motion). In practice, the cross-relaxation rate (NOE buildup rate) is usually compared to that for a known fixed distance and scaled according to

$$R_{1(\mathrm{ref})}/R_{1(ij)} = r_{ij}^6/r_{\mathrm{ref}}^6 \quad (2)$$

The use of such scaling to a reference distance is only valid if the unknown proton pair has the same correlation time as the reference proton pair. Thus, the choice of the reference fixed distance is important if the molecule under study exhibits internal motion in addition to the global motion in solution.

Even for a rigid structure with no internal motion, the two-spin approximation is only an approximation for a real multispin system in which relaxation through multiple pathways occurs simultaneously. In the presence of such multiple relaxation pathways, the two-spin approximation for any given proton–proton distance becomes less accurate as the mixing time is increased due to spin-diffusion effects from intervening spins (Kalk & Berendsen, 1976). However, these second-order processes have a finite lag or induction period, and their effects can be progressively reduced by sampling the cross-relaxation at shorter and shorter mixing times, under which conditions the observed initial rate two-spin approximation asymptotically approaches that for the true interproton distance.

Thus, the two major potential sources of error in NMR distance measurements are (a) variations in correlation times due to differential local motion and (b) incorrectly estimating the initial rate of cross-relaxation by sampling the system only at longer mixing times where significant spin diffusion has become established. In practice, the ability to sample the true initial cross-relaxation rates is limited by the signal-to-noise ratio of the very small crosspeaks at very short mixing times; i.e., the problem becomes one of spectrometer sensitivity. In general, it is not uncommon to use 100 ms as the first time point in attempting to measure initial cross-relaxation rates.

DNA contains at least two known fixed distances to which the rates for unknown distances can be scaled; these are the cytosine H5–H6 protons, separated by 2.44–2.48 Å (depending on the precise C–C–H bond angle and C–H bond length used), and the deoxyribose 2′H–2″H geminal protons, separated by 1.77–1.80 Å (depending on the precise C–H bond length used). In a rigid molecule in which both pairs of protons have the same correlation time, the initial rate of cross-relaxation for the 2′H–2″H should be 6.2–7.5 times faster than for the H5–H6 crosspeak. In their attempts to measure these two known reference distances in two DNA duplexes, Clore and Gronenborn (1984a,b) sampled the cross-relaxation at mixing times of 100–800 ms and observed 2′H–2″H cross-relaxation rates that were only 2.0 times faster than those for H5–H6. They attributed the 3-fold discrepancy from the rigid body predicted rate ratios to internal motion within the sugar, estimating a H5–H6 correlation time of 2.2–2.4 ns and a 2′H–2″H correlation time of 0.8 ns in a 6-bp duplex, and proceeded to measure all unknown base–base and base–1′H distances using the longer correlation time and all unknown sugar–sugar and sugar–base distances using the shorter correlation time.

The distances determined by these NMR methods are extremely important in that they constitute the only experimental input to DNA structure determination. It is our contention that renormalizing cross-relaxation rates is not a valid method of probing internal motion in polynucleotides, and we decided to reinvestigate the evidence for these claims of internal motion and shorter correlation times for sugar protons in short DNA duplexes.

Materials and Methods

DNA dodecamers containing the *Eco*RI and *Bcl*I restriction sequences, [d(CGCGAATTCGCG)]$_2$ and [d(GCCTGATCAGGC)]$_2$, respectively, were synthesized by using solid-phase phosphite triester techniques as previously described (Hare & Reid, 1986). The synthetic DNA samples were purified by chromatography in distilled water on a Sephadex G-25 column 120 cm in length. The lyophilized duplexes were dissolved in 0.4 mL of buffer containing 20 mM sodium phosphate, pH 7.0, and 50 mM NaCl. The DNA samples were repeatedly lyophilized to dryness and redissolved in 99.96% D_2O. Finally the sample was dissolved in 0.4 mL of 99.996% D_2O and transferred to a 5-mm Norell NMR tube. The DNA sample size was typically 24–28 mg, resulting in final concentrations of ca. 9 mM in duplex for the NMR studies.

The initial NMR experiments on the *Eco*RI duplex were performed on a home-built 500-MHz NMR spectrometer. Six NOESY spectra with mixing times of 50, 100, 200, 300, 500, and 700 ms were collected. Subsequent NMR experiments on both the *Eco*RI and *Bcl*I duplexes were acquired on a Bruker WM-500 spectrometer. For the *Eco*RI duplex, three additional NOESY spectra with mixing times of 15, 30, and 60 ms were obtained, and six NOESY spectra with mixing times of 30, 60, 90, 120, 150, and 180 ms were collected for the *Bcl*I duplex; sample temperature was maintained at 36 °C. For all of the above experiments, 1024 complex points in t_2 and 400 points in t_1 were collected with 32 scans per t_1 experiment being acquired, except for the 15-ms NOESY performed on the *Eco*RI duplex, where 64 scans per t_1 were collected. Each set of time-dependent NOESY spectra was collected within a single 4–5-day period without removing the sample from the spectrometer. The pure absorption NOESY spectra were collected by using the phase-sensitive method of States et al. (1982). In each NOESY spectrum the mixing period was randomly jittered over ±10% of the corresponding mixing time to filter out zero-quantum coherence transfer, with the exception of the 15-ms NOESY experiment, which was randomly varied between 12 and 18 ms of mixing. It is not essential to totally eliminate coherence transfer in phase-sensitive NOESY spectra since the antiphase coherence components self-cancel and alter the shape, but not the total intensity, of the multiplet NOESY crosspeak; it is, however, important to reduce the coherence contribution below the cross-relaxation intensity to avoid complications in integration of the crosspeak caused by negative components below the base-line plane.

Following collection, the acquired data were copied onto magnetic tape and transferred to a MicroVax II for processing using the FTNMR program (Hare Research Inc.). Both *Eco*RI data sets were zero-filled to 2048 points before Fourier transformation. For the initial phase-sensitive *Eco*RI NOESY spectra, no apodization was applied in the t_2 dimension since strong apodization can distort relative crosspeak intensities. For the subsequent shorter mixing time phase-sensitive NOESY experiments on *Eco*RI, exponential line broadening of 2 Hz was applied to decrease spurious noise resulting from less intense crosspeaks. The *Bcl*I data sets were multiplied by a window function in t_2 with a value of 1 for the first 624 points which smoothly fell to zero at 1024 points as a sine-bell-squared function to minimize truncation effects without affecting crosspeak volumes. After Fourier transformation,

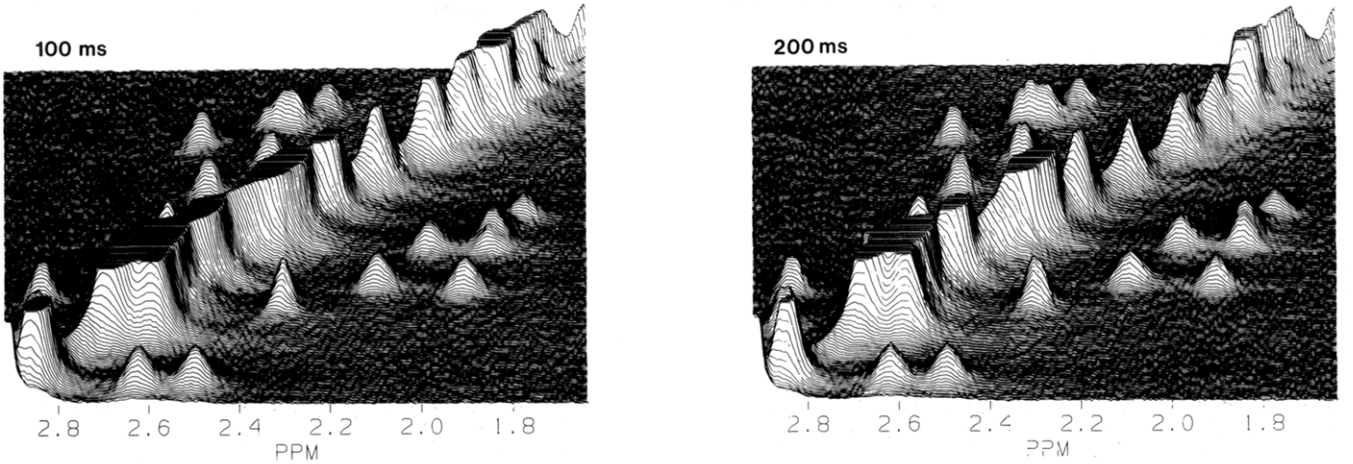

FIGURE 1: Stack plots of the phase-sensitive NOESY spectrum of $[d(CGCGAATTCGCG)]_2$ showing the 2′H–2″H geminal crosspeaks at 100- and 200-ms mixing times.

the data were phase-corrected to yield pure absorption line shapes.

In the t_1 dimension, the *Eco*RI data were zero-filled to 2048 complex points. In all cases, the data were multiplied by a window function with a value of 1 for the first 280 points which then decreased smoothly as a sinebell-squared function to zero at 400 points to minimize truncation effects on protons with longer T_2 relaxation. Fourier transformation and phase correction followed to complete the data processing.

The volume integrals for well-resolved H5–H6 and 2′H–2″H crosspeaks were then measured from the NOESY contour plots at each of the mixing times yielding the growth rate of each crosspeak. The FTNMR algorithm calculates peak volumes by summation of all points within an operator-specified ellipse surrounding the peak.

Results

The *Eco*RI duplex $d[(CGCGAATTCGCG)]_2$ and the *Bcl*I duplex $[d(GCCTGATCATGGC)]_2$ were chosen for this study on the basis of their relatively simple NMR spectra and the presence of several resolved 2′H–2″H and H5–H6 crosspeaks in their NOESY spectra. Figure 1 shows raw stack plots from unsymmetrized data on the 2′H–2″H crosspeaks in $[d(CGCGAATTCGCG)]_2$ at mixing times of 100 and 200 ms. Although the 2′H and 2″H chemical shifts are fairly similar and the crosspeaks are quite close to the diagonal, there are still several single crosspeaks that are sufficiently resolved to be reliably integrated. The most obvious point to be made from these data is that the crosspeak intensities do not increase at all between 100 and 200 ms, and hence the initial rate estimated from the 100-ms time point is obviously a gross underestimation of the true initial rate.

Figure 2 shows the corresponding 2′H–2″H crosspeaks in the NOESY spectra of $[d(GCCTGATCAGGC)]_2$ sampled at 30, 60, 90, and 120 ms. The geminal crosspeaks now show significant growth in the 30–60-ms period (but less than a linear doubling) and then taper off to a plateau intensity at 90 ms. The intensities of the geminal 2′H–2″H crosspeaks for C2 and A6, as well as those for the H5–H6 crosspeaks of C2 and C8, are plotted at 30-ms intervals out to 180 ms in Figure 3; the bars above and below the symbols in the graph are not error bars—they are the integrated intensities of the above-diagonal and below-diagonal crosspeaks in raw unsymmetrized data. Also shown in this plot are the "initial rates" for the 2′H–2″H and H5–H6 crosspeaks obtained by sampling the system only at 100 ms (open squares). The 2′H–2″H crosspeak intensity is 1.8 times that of the H5–H6 crosspeak

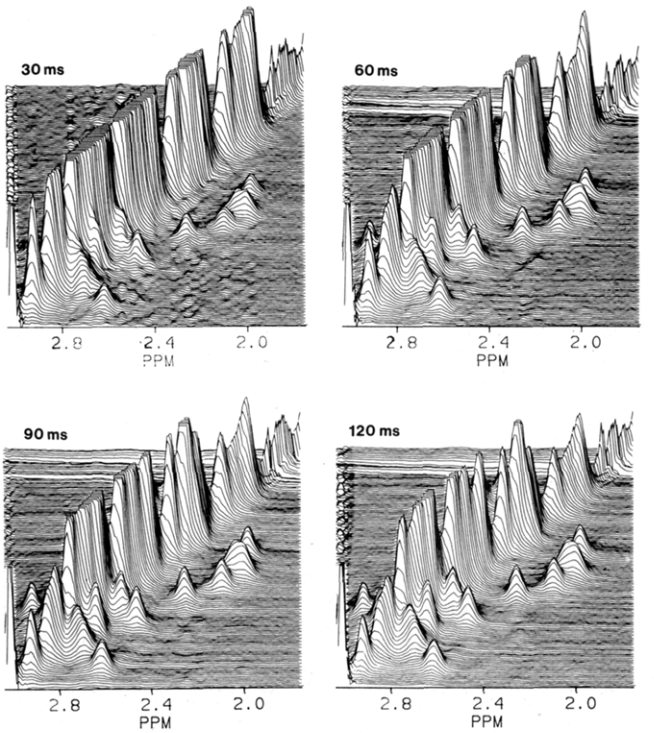

FIGURE 2: Stack plots of the 2′H–2″H crosspeaks in phase-sensitive NOESY spectra of $[d(GCCTGATCAGGC)]_2$ at mixing times of 30, 60, 90, and 120 ms.

at 100 ms, and it is apparent that, instead of growing, the 2′H–2″H intensity is actually beginning to decrease by 100 ms. Thus, the H5–H6 initial rate sampled with a single 100-ms time point slightly underestimates the true initial rate while the 100-ms 2′H–2″H initial rate seriously underestimates the true initial rate for this shorter distance. At sampling times of 30 ms the growth rate of the 2′H–2″H crosspeak increases to 3.2–3.6 times that of the H5–H6 crosspeak. Although this is undoubtedly a better estimate of the initial rate than the apparent rate at 100 ms, it is equally apparent that even at 30 ms one is not yet sampling the linear part of the true initial rate.

We next repeated these experiments on $[d(CGCGAATTCGCG)]_2$ with mixing times of 15, 30, and 60 ms. The raw data for the 2′H–2″H crosspeaks are shown in Figure 4, and the integrated intensities are plotted as a function of time in Figure 5. Visual inspection of the raw data reveals marked growth of the crosspeak intensities at all three time

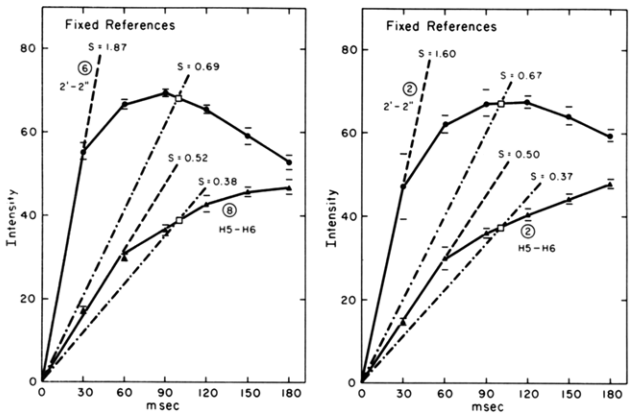

FIGURE 3: Plots of the time dependence of the NOESY intensities (volume integrals) for the 2′H–2″H crosspeaks (solid circles) of A6 and C2 and for the H5–H6 crosspeaks (solid triangles) of C8 and C2 in $[d(GCCTGATCAGGC)]_2$. The apparent initial rates are extrapolated as dashed lines with the slope value at the end of extrapolated line. Also shown, as open squares and dash-dot lines, are the apparent initial rate slopes obtained by sampling the system with 100 ms as the first time point. The hash marks above and below each data point are the above-diagonal and below-diagonal intensities in unsymmetrized spectra; they are not error bars.

points, with the intensity almost doubling between 15 and 30 ms. This result strongly suggests that the rate observed experimentally at 15 ms is now very close to the true initial cross-relaxation rate for geminal protons separated by 1.78 Å. The initial rate estimates for the geminal 2′–2″ protons of T7, T8, and C9 vary from ca. 14 to ca. 19 arbitrary volume units/s; more important is the observation that the experimentally measured 2′H–2″H cross-relaxation rate is now in the range 5.6–7.6 times faster than the experimentally measured H5–H6 cross–relaxation rate. The theoretical rate ratio for a rigid molecule with no internal motion, i.e., assuming that base protons and sugar protons have the same correlation time, is 6.2–7.5 (depending on the precise C–H bond lengths used). The results unequivocally demonstrate that spin diffusion, not internal motion, is responsible for the reduced apparent 2′H–2″H cross-relaxation rate at 100 ms. While protons separated by 2.4–2.5 Å cross-relax reasonably linearly up to 60 ms or so, protons separated by less than 1.8 Å cross-relax so rapidly that spin diffusion begins to cause deviations from linearity within less than 20 ms, and these deviations are quite serious by 100 ms.

Since we had already collected complete NOESY data sets on both duplexes down to quite short mixing times, we decided to analyze the early mixing data from other spectral regions containing intra- and interresidue crosspeaks corresponding to unknown distances to assess the reliability and precision of distance measurements in the 2–4-Å range. Figure 6 shows the raw data for the base to 2′H,2″H crosspeaks in $[d(CGCGAATTCGCG)]_2$ at 15, 30, and 60 ms. While the signal-to-noise ratio is a little scratchy at 15 ms, and the data are somewhat compromised by t_1 ridges, it is nevertheless apparent that resolved crosspeaks for the closer distances are well above the noise level and can be integrated reasonably reliably. The more pronounced crosspeaks in this region grow at approximately one-fourth the rate of the geminal 2′H–2″H crosspeaks and somewhat faster than the H5–H6 crosspeaks, i.e., they correspond to distances in the 2.2–2.3-Å range. Figure 7 shows a plot of the integrated intensities as a function of time (again the bars are the above/below-diagonal crosspeak intensities) for NOEs between the 1′H of A6 and the other protons in this sugar, as well as for the crosspeaks from T7–H6 and A6–H8 to sugar protons of the same or adjacent residue in the duplex $[d(GCCTGATCAGGC)]_2$. The initial slopes are indicated by extrapolated heavy dotted lines at the ends of which are the slope values (in arbitrary volume units per second) as well as the distances calculated from these slopes by scaling them to the H5–H6 slope using a value of 2.46 Å for the latter distance.

From the cross-relaxation rate plots, several noteworthy points emerge. Distances of less than 2.4 Å produce rate plots that begin to deviate from linearity by 60 ms. Such very close protons are unlikely to have intervening spins that contribute additional magnetization transfer, and the slopes invariably fall off with time. The slopes for distances in the 2.7–3.3-Å range tend to remain linear out to 60–90 ms. For distances in the 3.5–4.1-Å range, the slopes remain fairly linear out to 90–120 ms and then either fall off or increase (induction). While the reliability or precision of the slope measurements is partially reflected in the above-diagonal and below-diagonal bars bracketing each point, the tolerance in angstroms was also estimated by plotting the slopes corresponding to slightly larger or smaller distances. As shown in the upper plot of Figure 7, the sixth root is a rather forgiving lever; even for the rapidly cross-relaxing 1′H–2″H distance of 2.36 Å, the obviously nonlinear 60-ms intensity is still within the range 2.36 ± 0.15 Å, and it appears that the precision of distance measurements in the 2–3-Å range is well within this confidence level, i.e., a precision of ca. 5% or better in carefully collected data. For longer distances beyond 3 Å, the measurement precision remains in the 5–10% range, but the possible presence of intervening spins, and their precise location, reduce the accuracy of the distance estimates for these longer distances due to indirect second-order pathways. We have used error estimates of this type to establish the upper bounds and lower bounds

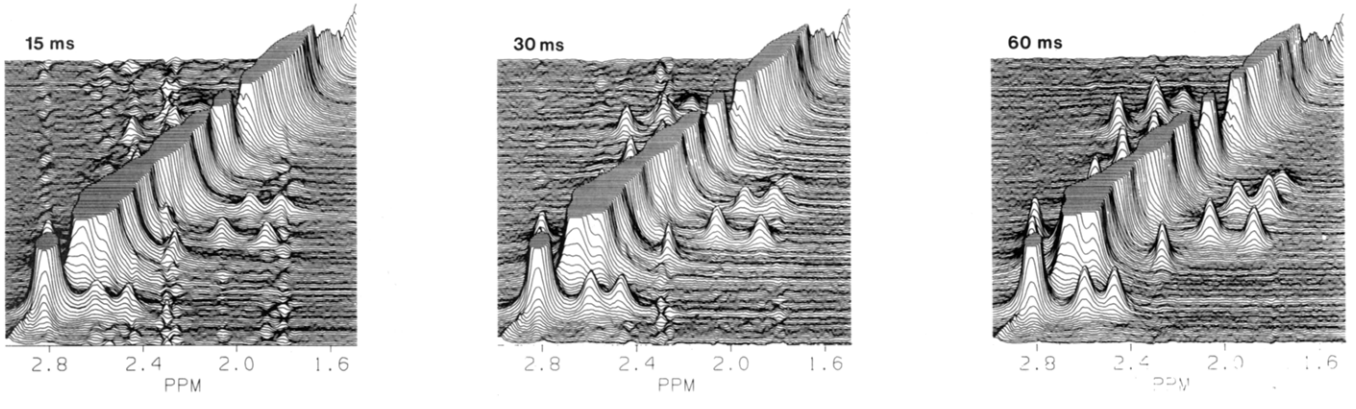

FIGURE 4: Stack plots of the phase-sensitive 2′H–2″H NOESY crosspeaks at $[d(CGCGAATTCGCG)]_2$ at mixing times of 15, 30, and 60 ms.

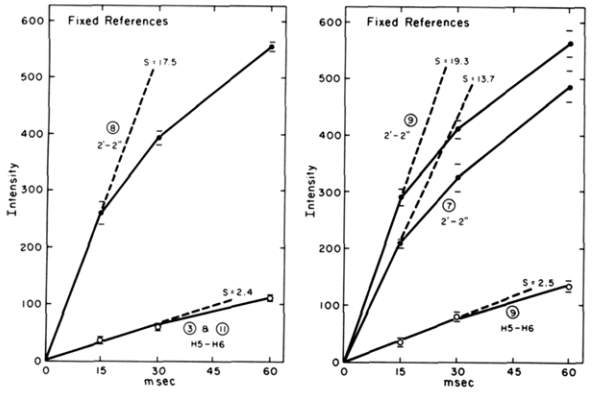

FIGURE 5: Plots of the time dependence of the NOESY intensities for the 2′H–2″H crosspeaks (solid circles) of T7, T8, and C9 and the H5–H6 crosspeaks of C3, C9, and C11 in $[d(CGCGAATTCGCG)]_2$ at 15, 30, and 60 ms. The H5–H6 crosspeaks of C3 and C11 are partially overlapped, and their combined intensity was divided by two. Extrapolated initial rates are shown as dashed lines labeled with the corresponding slope value in arbitrary volume units per second. The hash marks above and below each data point are the above-diagonal and below-diagonal intensities in unsymmetrized spectra.

for the input distances in the distance matrices that we use to calculate DNA structures (Banks et al., 1989; Nerdal et al., 1989). While the precision of the slope measurement may not necessarily reflect the accuracy of the calculated distance, it is worth returning to the measured slopes ranging from 14 to 19 s^{-1} for the 2′H–2″H geminal protons and using them to calculate the distances corresponding to the measured slopes for the H5–H6 protons (known to be 2.44–2.48 Å apart). Using a value of 1.78 Å for the 2′H–2″H distance, one obtains a maximal H5–H6 distance of 2.51 Å, i.e., 2.46 + 0.05 Å, and a minimal H5–H6 distance of 2.37 Å, i.e., 2.46 − 0.09 Å. It would appear that the accuracy of distance measurements in the 2.5-Å range is ±0.1 Å. This is approaching the amplitude of thermal fluctuations and is close to the range of precision to which the H5–H6 distance is actually known.

Discussion

We have, at 100 ms, reproduced the data of Clore and Gronenborn (1984a,b) and Gronenborn and Clore (1985) in which the 2′-H–2″H crosspeaks of DNA NOESY spectra appear to grow at only twice the rate of the H5–H6 crosspeaks, instead of the 6–7 times faster rate expected for a rigid molecule in which all proton pairs have the same correlation time. We have also demonstrated that their conclusion, namely that the sugars must have a 3-fold shorter correlation time due to nanosecond local motion to rationalize the slower apparent 2′H–2″H cross-relaxation rate, is wrong. When the data are sampled at mixing times shorter than 100 ms, the 2′H–2″H NOESY peaks (1.78 Å) do in fact grow at 6–7 times the rate of the H5–H6 crosspeaks (2.46 Å), as predicted for a rigid molecule in which the interproton vectors have the same correlation time. The data presented here convincingly show that, at 100 ms, the artifact in the apparent 2′H–2″H NOE buildup rate is due to spin diffusion, not local motion. While admittedly not the best method to probe local motion and measure correlation times, our results are entirely consistent with the absence of significant nanosecond local motion in the sugar moieties of short duplex DNA molecules in solution. We are currently synthesizing these same duplexes containing a single deuteron at the 2″ position of one of the internal sugars to more rigorously investigate sugar motion in DNA by deuterium solid-state motional methods that avoid the ad hoc assumptions of proton dipole-dominated systems. The errors in the previously published studies are caused by the very rapid cross-relaxation of the extremely close geminal 2′H and 2″H; at 1.78 Å these protons cross-relax over 80 times faster than H8–1′H protons separated by ca. 3.7 Å and attain almost instantaneous magnetization equilibrium. Thus, the loss of acceptor (2″H) magnetization to other proximal spins and back to the saturated donor proton (2′H) leads to greatly reduced magnetization of the acceptor at 100 ms in the one-dimensional truncated driven NOE experiments of Clore and Gronenborn (1984a,b). Our present results indicate that such artifacts can be almost eliminated by sampling the system at much earlier mixing times, under which conditions the fixed reference distances in the base and in the sugar scale perfectly normally as the inverse sixth power of their distances. Our results thus indicate that all proton–proton distances in short DNA duplexes, whether sugar–sugar, base–base, or sugar–base, can be determined by scaling the initial NOE buildup rate to the slope for the cytosine H5–H6 crosspeak; this is an important point in that 2′H–2″H NOESY crosspeaks are close to the diagonal and are usually unresolved and impossible to measure in more complex spectra of duplexes containing more than 14–16 unique nucleotides.

In light of our inability to detect differential motion in the sugar moieties of DNA by high-resolution proton NMR, it is worthwhile to survey the published claims in the literature that purport to demonstrate such local motion. The question of whether or not nanosecond local motions of significant amplitude exist in DNA is intimately related to the problem of collective motional deformations of the elastic filament and is a contentious area with many conflicting claims and reports. Data from Jardetzky's laboratory (Hogan & Jardetzky, 1980) claiming local motion on the basis of ethidium bromide induced effects on phosphorus relaxation could not be reproduced in Wilson's laboratory (Jones & Wilson, 1980; Wilson et al., 1981) and have been disputed by Schurr's laboratory (Allison et al., 1982), who claim the observed disappearance of the ^{31}P signal is merely a phase-separation artifact. Relaxation studies in James's laboratory (Bendel et al., 1982; Keepers & James, 1982) were interpreted in terms of large amplitude (50–70°) jumps on the nanosecond time scale, and models were proposed to mimic these large local motions (Keepers & James, 1982). They also reported a phosphorus T_2 value for a supercoiled plasmid that was over 500 times longer than previously re-

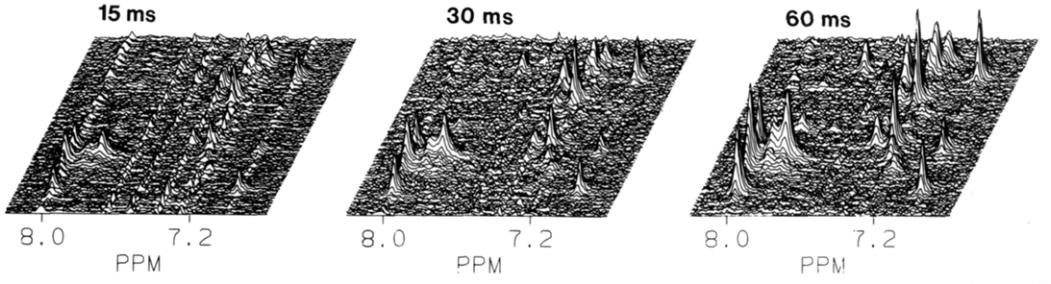

FIGURE 6: Stack plots of the base H8/H6 to 2′H, 2″H crosspeaks region in $[d(CGCGAATTCGCG)]_2$ at mixing times of 15, 30, and 60 ms.

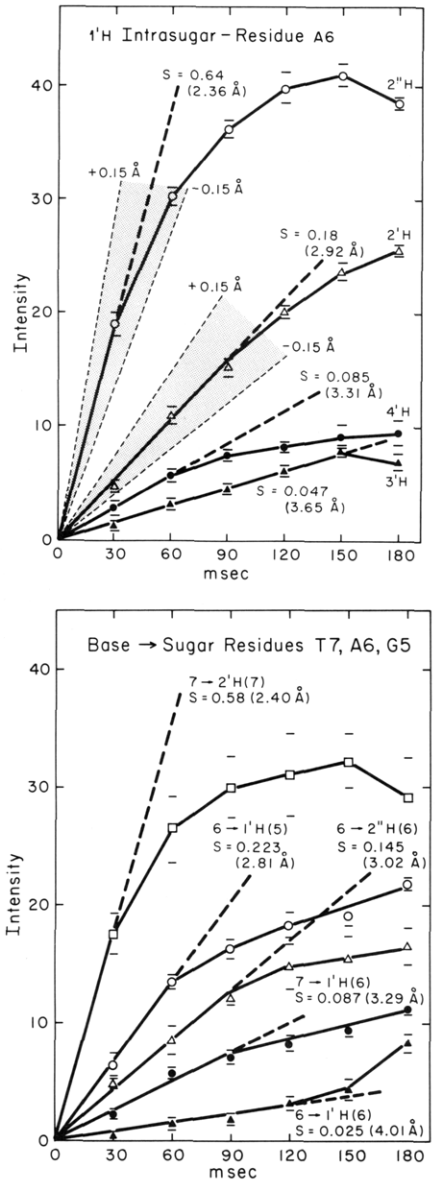

FIGURE 7: (Top) Time-dependent NOESY intensities for the crosspeaks between 1'H and the other sugar protons (2'H, 2"H, 3'H, and 4'H) in the deoxyribose ring of residue A6 in $[d(GCCTGATCAGGC)]_2$. The hash marks are the above-diagonal and below-diagonal intensities for each time point in unsymmetrized spectra. Each initial rate is extrapolated as a dashed line, at the end of which is the slope value and the corresponding distance obtained by scaling the slope to the slope of the cytosine H5–H6 crosspeak. Also shown as a stippled gray zone for the 1'H–2"H and 1'H–2'H crosspeaks are the slopes corresponding to distances of ±0.15 Å from the observed slope. (Bottom) Some representative NOE buildup rates for intraresidue and interresidue base to sugar proton crosspeaks in $[d(GCCTGATCAGGC)]_2$. Note that A6 H8–G5 1'H cross-relaxes 2.6 times faster than T7 H6–A6 1'H and 8.9 times faster than A6 H8–A6 1'H, indicating marked variation in anomeric proton to base proton distances in this duplex.

ported values for much smaller DNAs. This result, as well as their earlier interpretation of relaxation data in terms of large-amplitude local angular motion, has been called into question by Langowski et al. (1985). In their relaxation analysis Keepers and James (1982) ignore the contribution of collective torsional deformations of the DNA elastic filament. When these collective motions are properly taken into account, all of the published relaxation data can be fitted with collective motions with an RMS amplitude of 6–7°, leaving local motion amplitudes of only 7–10° (Langowski et al., 1985; Schurr & Fujimoto, 1988). Since $(0.93)^{1/6} \approx 0.99$, neither of these motions will affect the accuracy of NOE distance measurements by more than 1–2%, and these errors will be further reduced by scaling the relaxation rates to an internal fixed distance. Thus, there currently exists no hard evidence for large-amplitude nanosecond local motions in either the sugars or bases of DNA.

If large amplitude motions do exist in DNA, they cannot occur on the 1–500-Hz time scale in that we do not observe line splitting in 500-MHz DNA spectra. Present high-resolution methods are insensitive to microsecond motions but planned deuterium solid-state studies should cast light on this intermediate time scale. However, our previous distance geometry studies on DNA structure (Hare & Reid, 1986) indirectly argue against large-amplitude motions on this time scale. In such motion the closest approach for all proton pairs would be heavily emphasized in distance measurements due to the inverse sixth power dependence of dipolar cross-relaxation. This would inevitably result in "impossible structures" that could not satisfy the known C, N, O covalent angles and bond lengths—yet the structures generated by distance geometry satisfy the measured NOE distances without any noticeable covalent strain.

Returning to interproton distance measurements, as mentioned above the principal use of these distances is to generate structures, whether by ab initio distance geometry calculations or by refinement of classical averaged coordinates from fiber diffraction data using distance-constrained energy minimization/molecular dynamics methods. It follows quite logically that if the starting distances are wrong, the resulting structures that satisfy these distances will be wrong. The fundamental misconception concerning 3-fold shorter correlation times for sugar protons introduces systematic errors in the range 0.4–0.7 Å in the majority of proton distances (sugar–sugar and sugar–base distances) measured by the methods currently recommended in the literature (Clore & Gronenborn, 1984; Gronenborn & Clore, 1985). For example, consider a H8–2"H or a 1'H–2"H NOESY crosspeak that grows at an initial rate exactly equal to the reference cytosine H5–H6 initial rate. Since the bases and sugars have the same correlation time, the H8–2"H or 1'H–2"H distance is obviously 2.46 Å. However, processing this same experimental data on the assumption of 3-fold faster internal motion of sugar protons to scale the slopes would result in a calculated H8–2"H or 1'H–2"H distance of 2.05 Å. For other proton pairs with initial rate slopes of 30% of the H5–H6 slope (a true distance of 3.0 Å), the calculated distance would be 2.50 Å, and for observed slopes of 5.4% of the H5–H6 rate (a true distance of 4.0 Å) the calculated distance would be 3.33 Å, i.e., an error of 0.67 Å if the incorrect, shorter sugar correlation time is used in processing the data. It should be noted that the large majority of intranucleotide and internucleotide proton–proton distances involve at least one sugar proton, and if scaled by this method, all these distances will contain systematic errors of this magnitude. Because many structures reported for DNA in solution have been refined to agree with incorrect distances scaled in this way (Clore & Gronenborn, 1984a,b, 1985a,b; Gronenborn et al., 1984; Clore et al., 1985, 1986, 1988; Gronenborn & Clore, 1985; Nilsson et al., 1986; Nilges et al., 1987a,b), the relevance and validity of these structures, as well as their ability to shed light on the process of DNA recognition, are suspect. Furthermore, even in the absence of significant sugar and base nanosecond internal motions, some of the two-spin approximated 60–90-ms initial rate distance estimates are distorted by spin diffusion, and these errors are only revealed by back-calculation simulations of the NOESY spectra

that include multiple spin pathways (Banks et al., 1989; Nerdal et al., 1989). No matter how the structures are arrived at, structures that satisfy incorrect distances cannot be correct.

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