Local Dynamics of the CpG Step in a DNA Crystal

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The inconsistent nature of the samples studied by different techniques makes it difficult to compare the results of experiments done on biological macromolecules. Solution-state NMR samples are dissolved in appropriate buffer systems and are studied in the liquid state. X-ray diffraction studies require a single-crystal sample, and the environment of these crystals is determined by the solvent combination necessary for crystal growth. Solid-state NMR studies of biological macromolecules are usually done on powder samples of variable relative humidity. Even for a DNA sample as well-studied as the Dickerson dodecamer (d[CGCGAATTCGCG]$_2$),[1−9] it is difficult to understand the relevance of one data set to another obtained with a different technique.

To address this question, we crystallized a C9 furanose-labeled sample that has been previously studied via solid-state ^{2}H NMR as an amorphous powder.[7] This sample contains a [2″-^{2}H]-2′-deoxycytidine at the C9 position of the Dickerson dodecamer. The synthesis of this sample has been described previously.[7] The SSNMR studies of this sample show that the deuterium-labeled furanose ring undergoes unusual, large-amplitude dynamics at increased hydration levels. These large-amplitude dynamics are unusual as they occur only at the furanose rings of the C3 and C9 nucleotides.[8] This is significant as the C3 nucleotide is next to and the C9 nucleotide across from the G4 nucleotide, the site where the EcoRI restriction endonuclease binds and cleaves. This unusual pocket of increased amplitude dynamics near the enzyme-cutting site may indicate that DNA dynamics have a role in protein−DNA recognition.

The DNA sample was crystallized from 1,2-methylpentanediol in the presence of spermine as described in the literature.[3,10] To obtain enough crystallized DNA for a deuterium NMR study (∼20 mg), a larger-volume method was required. The sitting-drop method of crystallization allows well volumes of 80 μL.[11] Assuming an 80% recovery of DNA from each well, the total sample prepared for NMR studies was ∼12 mg.

An X-ray diffraction data set of a large single crystal from a 30% MPD hanging drop was collected on a Raxis II area detector. The details about data collection and refinement statistics may be found in Table 1. The space group was found to be $P2_12_12_1$, the same as that reported by the Dickerson group.[2] The unit cell parameters were identical to the Dickerson structure (a = 40.4 Å, b = 24.9 Å, c = 66.2 Å), and refinement of the Dickerson model using the diffraction data collected from our crystal showed it to be isomorphous with Dickerson's.

The solid-state ^{2}H NMR was done on a home-built spectrometer with a deuterium resonant frequency of 76.76 MHz (Gladden and Drobny, unpublished results). The line shapes were obtained using a simple quadrupolar echo pulse sequence. The line shape of the powder sample was achieved with signal averaging of 320 000 scans and a recycle delay of 200 ms. The line shape of the crystal sample was obtained with signal averaging 1 280 000 scans and a recycle delay of 300 ms. The quadrupolar line shapes were calculated using the program MXQET.[12]

Figure 1 shows these line shapes and simulations. Figure 1A shows the spectrum of the powder sample hydrated to 80% relative humidity, and Figure 1B shows the spectrum of the crystalline sample. The large center peak in the crystalline spectrum is the result of residual HDO in the mother liquor present in the sample holder. This solution must be present to maintain the integrity of the crystalline sample. The comparison of the crystalline sample to the 80% RH powder is appropriate as the Dickerson X-ray results propose a water content of 11 waters per base,[3] and gravimetric analysis of the powder sample at 80% RH provides a water content of 13 waters per base.

These line shapes have been simulated using Brownian diffusion in a double well potential U, where $U = U_o/2(1 - \cos 2\theta)$ and U_o is the barrier height. This model has been described in detail elsewhere.[13] The best fit for the C9 furanose-labeled sample described in this communication is found when the barrier is set to 5 kcal/mol and the cone-half angle is 38°. This simulation is shown in Figure 1C. Figure 1D shows the simulation of a small amplitude libration (±8°) for comparison.

Progressive saturation experiments are done to determine the average Zeeman relaxation time of samples with low signal-to-noise. This experiment was done on the crystalline sample by measuring the spin−echo intensity of data sets collected at magnetization recovery times of 1, 10, 25, 50, 75, and 100 ms. The $\langle T_{1z} \rangle$ was determined to be 20 ± 11ms. While this error is significant, the high end value of 31 ms is still well below that for sugar rings experiencing only small amplitude motion (∼100 ms[6,7]). However, this relaxation time of 20 ms is in the same range as that determined for the C9-labeled powder sample at 80% RH ($\langle T_{1z} \rangle$ = 25 ms).[7] The similarities shown in both the line shapes and the relaxation times of these two samples suggest that the dynamics in the crystal are the same as those observed in the powder sample.

X-ray crystallography and high-resolution NMR of the "Dickerson sequence" provide similar structures showing sequence-dependent variations including differences in sugar ring conformations between nucleotides.[1−4] However, X-ray, high-resolution

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(10) DNA solutions of 20 mg/mL were made in a 2.78 mM solution of magnesium acetate buffer at pH 7.4. The spermine solutions were prepared to ensure a concentration of one mole of spermine per eight base pairs of DNA. All solutions were made with deuterium-depleted water to decrease the presence of a background HDO signal in the NMR spectrum. Crystals were observed in hanging drop vapor diffusion chambers with 30 and 40% MPD.
(11) Forty-eight wells of 20 mg/mL DNA in 30% MPD were prepared. The crystals grown in these wells were smaller in size than those grown as hanging drops but still of quality crystalline morphology. After eight weeks, crystals were harvested from 38 of the 48 wells. The crystals plus mother liquor from the wells were transferred to a kel-f NMR sample holder.
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Table 1. Data Collection and Refinement Statistics

maximum resolution (Å)	2.1
measured reflections	6616
unique reflections	3154
completeness (%)	67.8
R_{merge}[a]	0.05
resolution limits for refinement (Å)	10.0−2.1
number of reflections in working set	2767
number of reflections in test set	338
R-factor	0.219
free *R*	0.308

[a] On intensities.

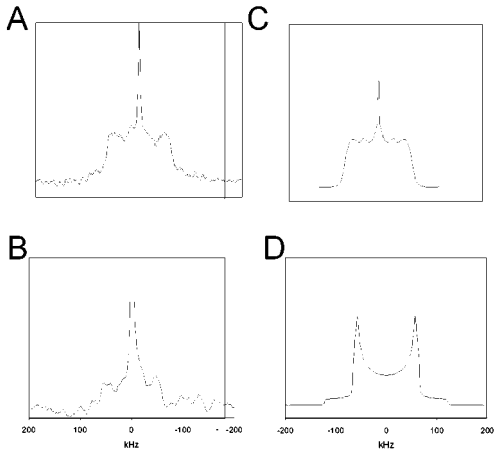

Figure 1. ^{2}H NMR line shapes and simulations. A and B show the powder and crystalline line shapes, respectively. C and D show simulations for large-amplitude (±38°) and small-amplitude dynamics (±8°), respectively.

NMR, and solid-state NMR all show different results for the dynamics of the sugar rings. As already discussed, the solid-state NMR studies show large-amplitude dynamics at the C3 and C9 furanose rings. However, the crystallographic segmented rigid body analysis of Holbrook and Kim reports larger-amplitude dynamics in the backbones and sugars than in the bases overall, but they do not observe any large-amplitude dynamics in the sugar rings.[5] This is not surprising since detailed structural information about hydrogen atoms is only available from X-ray crystallography experiments with 1.2 Å or better resolution. Thus, in this X-ray study, the dynamics of the sugar rings is discussed in terms of the carbon atoms. According to the theory of pseudorotation, the motion of the ring carbons perpendicular to the plane of the ring may not be greater than 0.4 Å, and thus large amplitude motions do not occur at the carbon atoms.[14] However, proton displacements can vary more dramatically; if the C−H bond-length is assumed to be 1.1 Å, then the proton displacement for a half-angle of libration equal to ±38° is equal to 1.35 Å, while a half-angle of libration equal to ±8° displaces the proton by only 0.31 Å. Both of these motions, which have significantly different proton displacements, can occur with only a small (0.4 Å) displacement of the carbon atom. Thus, the large-amplitude motion of the C−D bond observed in the solid-state NMR studies is not seen in the X-ray studies.

NMR scalar coupling analysis reports larger amplitude dynamics in the C3 and C9 furanose rings over the sugars of other nucleotides; however, the model described for these dynamics does not match that observed using solid-state ^{2}H NMR.[15] The small magnitudes of the scalar couplings and the presence of cross relaxation effects may be responsible for this discrepancy.[16] Another high-resolution NMR method for determining dynamics is model-free relaxation analysis.[17] This method assumes that the internal motions under investigation occur on time scales that are very different from that of the overall molecular motion. This is not generally the case for short DNA duplexes, and thus this method is not accurate for quantifying these furanose ring dynamics.

However, the broad line width in solid-state ^{2}H NMR (∼200 kHz) permits the investigation of a wide range of molecular motions. In addition, the presence of a quadrupolar moment and site-selective isotopic labeling allows solid-state ^{2}H NMR studies to provide information about the dynamics of an individual site in a macromolecule. These advantages make solid-state ^{2}H NMR an accurate technique for measuring furanose ring dynamics in DNA.

Using solid-state ^{2}H NMR we have shown that the C−D bonds at the C2′ position of the C9 furanose ring in both the crystalline and powder samples undergo large amplitude (±38°) dynamics. The characterization of these dynamics in both the crystalline and powder forms provides an opportunity to directly compare results from solid-state NMR and X-ray crystallography.

The results of this study, coupled with an understanding of the limitations of each of the techniques involved, show that using X-ray crystallography and solid-state ^{2}H NMR together may prove to be very effective in the effort to understand both the structure and dynamics of biological macromolecules.

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