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Furanose Dynamics in the *HhaI* Methyltransferase Target DNA Studied by Solution and Solid State NMR Relaxation

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Abstract

Both solid state and solution NMR relaxation measurements are routinely used to quantify the internal dynamics of biomolecules, but in very few cases have these two techniques been applied to the same system and even fewer attempts have been made so far to describe the results obtained through these two methods through a common theoretical framework. We have previously collected both solution ¹³C and solid state ²H relaxation measurements for multiple nuclei within the furanose rings of several nucleotides of the DNA sequence recognized by *HhaI* methyltransferase. The data demonstrated that the furanose rings within the GCGC recognition sequence are very flexible, with the furanose rings of the cytidine which is the methylation target experiencing the most extensive motions. In order to interpret these experimental results quantitatively, we have developed a dynamic model of furanose rings based on the analysis of solid state ²H line shapes. The motions are modeled by treating bond reorientations as Brownian excursions within a restoring potential. By applying this model, we are able to reproduce the rates of ²H spin-lattice relaxation in the solid and ¹³C spin-lattice relaxation in solution using comparable restoring force constants and internal diffusion coefficients. As expected, the ¹³C relaxation rates in solution are less sensitive to motions that are slower than overall molecular tumbling than to the details of global molecular reorientation, but are somewhat more sensitive to motions in the immediate region of the Larmor frequency. Thus, we conclude that the local internal motions of this DNA oligomer in solution and in the hydrated solid state are virtually the same, and we validate an approach to the conjoint analysis of solution and solid state NMR relaxation and line shapes data, with wide applicability to many biophysical problems.

Keywords

solid state deuterium NMR; solution state ¹³C NMR; DNA; deoxyribonucleic acid; methyltransferase; dynamics; simulations

I. Introduction

Despite the obvious importance of motion to the functions of proteins¹⁻⁹ and nucleic acids¹⁰⁻¹², quantitatively defining the role that internal dynamics play in biological processes remains a challenging task due to the complex nature of biomolecular motions. Functionally relevant internal motions range from single bond torsions (occurring in the ps time scale) to

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long range structural rearrangements (ms-s and even longer). In order to characterize dynamics comprehensively, we must account for molecular motions with rates spanning at least 10 orders of magnitude and perhaps more. However, it is well-known that different dynamic spectroscopies display variable sensitivity to different rates of motion. Therefore, to rely upon a single type of spectroscopic measurement runs the risk of obtaining an incomplete or even incorrect description of internal molecular motions. The best way to accurately characterize all functionally relevant molecular motions is to use more than one spectroscopy to probe a broad range of dynamic rates. Although a number of possible combinations of spectroscopies exist, solution and solid state NMR are a natural combination because they cover different regions of the motional spectrum in a complementary fashion and share a common theoretical framework, being essentially the same spectroscopy applied to different sample conditions. Indeed, the concerted use of solid state and solution NMR dynamic methods has been strongly recommended¹³, but to date there have been few if any sustained efforts to combine these two spectroscopies to study the same biological processes.

We have previously applied both NMR techniques to study the local motions of the furanose rings in the DNA recognition site for *HhaI* methylase^{14, 15}.

Dynamics plays a critical functional role in this system because the DNA undergoes extensive structural rearrangement to accommodate interactions with the enzyme and to expose the modification site¹⁶. Our experimental work demonstrated that the furanose rings within the GCGC recognition sequences are structurally labile a priori, i.e. experience considerable motions in the free DNA, and that the furanose ring of the cytidine which is the methylation target interconverts between C2' and C3' endo sugar conformations without traversing a significant energy barrier. These atypical motions may be associated with the structural rearrangements that occur upon binding of the DNA to the protein methylase, and are thus related to function.

But we also reported¹⁷ that while solid state NMR line shapes vary greatly from furanose ring to furanose ring for nucleotides within and adjacent to the GCGC moiety, and although furanose ²H relaxation times vary across the same GCGC recognition site by as much as 400%, solution ¹³C relaxation times obtained from the same structural sites differ by less than 10%. It seems counter-intuitive to assume that these measurements indicate that a molecule is internally more rigid in solution than in the hydrated solid state, so a more detailed analysis is required.

In this manuscript, we seek to reconcile ¹³C solution relaxation measurements with static ²H relaxation and line shape data by introducing a theoretical model of furanose motion that reproduces both sets of results in a self-consistent manner. First, we review the theoretical framework used to analyze dynamically modulated solid state NMR line shapes, and to analyze both solid state and solution NMR relaxation rates. Within this framework, we then quantitatively compare solid state and solution NMR studies of the two nuclei in the C2'-H2'' moiety of cytosine C6 and C8 within the furanose rings in the central GCGC sequence of the DNA dodecamer 5'-d(GATAGCGCTATC)₂. In order to analyze the data from both types of spectroscopy conjointly, we model furanose motions as diffusion in a restoring potential whose parameters are determined by fitting the deuterium line shapes. We use this model to simulate the solid state ²H spin-lattice relaxation rates and the solution state ¹³C spin-lattice relaxation rates. Based on these calculations, we explore the resemblance of localized internal motions in amorphous solid, hydrated DNA and the motions of the same DNA in solution. Finally, we discuss the extent to which solution NMR relaxation and solid state NMR relaxation/line shape measurements are sensitive to local and global motions of the furanose rings of DNA and to motions in the us-ns time regime in general.

II. Theoretical Framework

Our approach to studying internal biomolecular dynamics by a combination of solid state/solution NMR has three experimental components: (1) solid state deuterium NMR line shape analysis, and the analyses of (2) solid state NMR and (3) solution NMR relaxation rates. We want to determine the degree to which the internal motions in a DNA molecule in solution resembles those occurring in essentially the same molecule in the hydrated solid state, and thus the degree to which both spectroscopies can be used in a unified way to study dynamics. Analysis of the line shape yields detailed information on the nature of internal molecular motions uncomplicated by the occurrence of overall molecular tumbling. In general, the ^2H line is simulated using a small number of models of molecular motions; these models must also simulate the solid state relaxation data in order to be considered valid. Once the solid state NMR line shape and relaxation data are modeled successfully, testing the consistency of the model against solution NMR data determines the extent to which the local internal dynamics observed in hydrated, solid DNA exists and is observable in the same molecule in solution. This section consists of a brief overview of the theoretical framework used to simulate NMR line shapes and relaxation rates both in the solid state and in solution.

A) Static Solid State ^2H NMR Line Shape Theory

The theory of the dynamically modulated solid state deuterium line shape is well documented¹⁸ and will be described only briefly here. A static ^2H NMR line shape is measured using a quadrupolar echo experiment, where two 90° pulses with relative phase shift of 90° degrees and separated by a time duration τ_1 are applied to the deuterium spin system. As a result of this pulse sequence, a quadrupolar echo is formed at a time τ_2 after the second pulse. For this paper, the simplest theory suffices, which accounts for a single local molecular motion that modulates in time the orientation of a particular molecular structural moiety relative to a laboratory frame, (L).

To describe line shape modulation by a single internal motion, at least three reference frames are used. The static ^2H solid state line shape is dominated by the interaction of the nuclear quadrupole moment with external electric field gradients (EFGs). The EFG tensor can be approximated to be uniaxial for deuterons bonded to aliphatic carbons and its principal axis system (P) is defined so that the z axis is parallel to the C-D bond axis (Figure 1). Internal motions modulate the relative orientation of the P frame and the L frame. This is mathematically described using a third frame, designated C, which is defined relative to the molecular framework. The orientation of the C frame depends upon the nature of the motion. In a polymethylene chain, conformational changes between rotational isomeric states require that the z axis of the C frame is parallel to the C-C bond. A similar definition exists for motions of a cyclic system like a furanose ring and will be discussed in section III below. The C frame used in this study to describe motions of the furanose ring and the relationship of the C frame relative to the L frame are both shown in Figure 1.

Internal molecular motions modulate the solid angle $\Omega_{PC}=(0, \theta_{PC}, \varphi_{PC})$ which quantifies the mutual orientation of the P and C frames. As a result of the internal motion, this solid angle becomes time dependent, i.e. $\Omega_{PC}(t)=(0, \beta_{PC}(t), \gamma_{PC}(t))$. In a polycrystalline state, the line shape is averaged over all orientations of the sample; thus, the solid angle $\Omega_{CL}=(0, \theta_{CL}, \varphi_{CL})$ that relates the C frame to the L frame is distributed randomly. With these conventions, the static ^2H line shape is a function of the frequency ω and the pulse intervals τ_1 and τ_2 according to:

$$I(\omega, \tau_1, \tau_2) = \int_0^{2\pi} d\varphi_{CL} \int_0^\pi d\theta_{CL} \sin\theta_{CL} I(\omega, \tau_1, \tau_2, \varphi_{CL}, \theta_{CL}) \quad (1)$$

where the orientation-dependent line shape is the Fourier transform of the time domain response $m(t)$:

$$I(\omega, \tau_1, \tau_2, \Omega_{CL}) = \text{Re} \int_{-\infty}^{+\infty} m(t) e^{-i\omega t} dt = \text{Re} \sum_k \frac{b_k}{\lambda_k - i\omega} \quad (2)$$

In equation 2 $m(t)$, which is the transverse magnetization corresponding to the $m=1$ to $m=0$ transition is defined by:

$$\begin{aligned} m(t) &= e^{(t+\tau_2)A} e^{\tau_1 A^*} m(0) = T e^{\lambda(t+\tau_2)} T^{-1} \pi T^* e^{\lambda^* \tau_1} (T^*)^{-1} \pi m_0 \\ &= \sum_{j,k,l,m,n} T_{jk} e^{\lambda_k(t+\tau_2)} T_{kl}^{-1} T_{lm}^* e^{\lambda_m^* \tau_1} (T^*)_{mn}^{-1} m_{0,n} = \sum_k b_k e^{\lambda_k t} \end{aligned} \quad (3)$$

where $A=i\omega+\pi$, $A^*=-i\omega+\pi$ and the matrices T and T^* diagonalize A and A^* respectively, i.e. $T^{-1}AT=\lambda$ and $(T^*)^{-1}A^*T^*=\lambda^*$. The matrix π is composed of site exchange rates, and ω is a diagonal matrix with non-zero elements ω_i that are the orientation dependent frequencies:

$$\omega_i = \frac{3}{4} \frac{e^2 q Q}{\hbar} \sum_{a=-2}^{+2} D_{0,a}^{(2)}(\Omega_{PC}^{(i)}) D_{a,0}^{(2)}(\Omega_{CL}) \quad (4)$$

The subscript i in ω_i and the superscript (i) in $\Omega_{PC}^{(i)}$ denote the i^{th} of N structural sites located along the trajectory of motion. Specifically $\Omega_{PC}^{(i)}$ is the solid angle that relates for the i^{th} site the P frame and the C frame, and Ω_{CL} is the solid angle that relates the C frame to the L frame. The quadrupolar coupling constant $QCC = e^2 q Q / \hbar$ is a known quantity and varies only slightly among aliphatic deuterons; it can be approximated to be 170 kHz. The analogous equation for the magnetization corresponding to the $m=0$ to $m=-1$ transition can be obtained by negating the frequency expression in equation 4. In order to use equation 1 to calculate a dynamically averaged line shape, the form of the exchange matrix π must be determined. The form of π used to describe local furanose ring motions will be described in section III.

B) Solid State ^2H Relaxation Theory

The correlation function for single axis motion in a solid can be defined as follows¹⁹:

$$\begin{aligned} C_m(t) &= \langle D_{0m}^{(2)*}(\Omega_{PL}(0)) D_{0m}^{(2)}(\Omega_{PL}(t)) \rangle = \\ &\sum_{a,a'=-2}^{+2} \langle D_{0a}^{(2)*}(\Omega_{PC}(0)) D_{0a'}^{(2)}(\Omega_{PC}(t)) \rangle D_{am}^{(2)*}(\Omega_{CL}) D_{a'm}^{(2)}(\Omega_{CL}) = \\ &\sum_{a,a'=-2}^{+2} D_{am}^{(2)*}(\Omega_{CL}) D_{a'm}^{(2)}(\Omega_{CL}) d_{0a}^{(2)}(\beta_{PC}) d_{0a'}^{(2)}(\beta_{PC}) \langle e^{ia\gamma_{PC}(0)} e^{-ia'\gamma_{PC}(t)} \rangle \end{aligned} \quad (5)$$

where $\langle \rangle$ denotes an ensemble average. As in section IIA, it is assumed in equation 5 that the electric field gradient (EFG) tensor is axially symmetric. All other conventions are the same as in section IIA: i.e. the solid angle $\Omega_{PC}(0, \beta_{PC}, \gamma_{PC})$ defines the mutual orientation of the principal axis system (P) of the EFG tensor and a frame fixed to the molecule (C). It is possible to select the axis system C such that only the angle γ_{PC} is time dependent as a result of changes of the molecular structure. For convenience we simplify the notation as follows: $\gamma_{PC}(0) = \gamma_0, \gamma_{PC}(t) = \gamma$.

The correlation function $\langle e^{ia\gamma_0} e^{-ia'\gamma} \rangle$ is given by

$$\Gamma_{a,a'}(t) = \langle e^{ia\gamma_0} e^{-ia'\gamma} \rangle = \int_{-\theta}^{+\theta} d\gamma \int_{-\theta}^{+\theta} d\gamma_0 e^{-ia\gamma_0} e^{-ia'\gamma} P(\gamma, t | \gamma_0) W(\gamma_0) \quad (6)$$

where $P(\gamma, t | \gamma_0)$ is the probability that the system is oriented at γ_t at time t conditional upon being at γ_0 at $t=0$. $W(\gamma_0)$ is the *a priori* probability of the system being at γ_0 . The form of the conditional probability depends, like the exchange matrix π , on the detailed nature of the motion of the furanose ring; this is treated in section III. The spin-lattice relaxation rate depends on the orientation of the crystallite to the laboratory frame given by the angle Ω_{CL} . A convenient simplification is to calculate the “powder averaged” spin-lattice relaxation rate. This can be carried out at the level of equation 5 by averaging the correlation function over the solid angle Ω_{CL} :

$$\bar{C}_m(t) = \frac{1}{8\pi^2} \int d\Omega_{CL} \sum_{a,a'=-2}^{+2} D_{am}^{(2)*}(\Omega_{CL}) D_{a'm}^{(2)}(\Omega_{CL}) d_{0a}^{(2)}(\beta_{PC}) d_{0a'}^{(2)}(\beta_{PC}) \langle e^{ia\gamma_{PC}(0)} e^{-ia'\gamma_{PC}(t)} \rangle \quad (7)$$

By applying to equation 7 the orthogonality condition for Wigner rotation matrices:

$$\int d\Omega_{CL} D_{MN}^{(L)}(\Omega_{CL}) D_{mn}^{(l)}(\Omega_{CL}) = \frac{8\pi^2}{2l+1} \delta_{Ll} \delta_{Mm} \delta_{Nn} \quad (8)$$

we obtain the following expression for the “powder-averaged” correlation function:

$$\bar{C}(t) = \frac{1}{5} \sum_{a=-2}^{+2} d_{0a}^{(2)}(\beta_{PC}) d_{0a}^{(2)}(\beta_{PC}) \Gamma_{aa}(t) \quad (9)$$

which is no longer dependent on the index m . Using equation 9, the “powder-averaged” spin-lattice relaxation rate is:

$$\frac{-\rho}{T_1} = \frac{1}{-\rho} = \frac{\omega_Q^2}{3} (J(\omega_0) + 4J(2\omega_0)) \quad (10)$$

In this expression, the spectral densities are Fourier transforms of the correlation function in equation 9 at particular frequencies, and are thus independent of the index m :

$$J(\omega) = \text{Re} \left[\int_{-\infty}^{+\infty} dt e^{-i\omega t} \bar{C}(t) \right] \quad (11)$$

C) Solution ^{13}C Relaxation Theory

Solution NMR relaxation rates are expressed in this paper according to well-established formalisms²⁰. Here, the simplest case of a single internal motion is assumed, as defined and described previously by the reorientation of the P frame relative to the C frame. An additional coordinate frame D must be defined to describe overall tumbling of the macromolecule. For duplex nucleic acids consisting of only twelve base pairs, rigid rod tumbling is a good approximation and the z axis of the D frame (which can be associated with the principal value

of the diffusion tensor $D_{\parallel}$) is parallel to the helix axis. The correlation function thus has the form:

$$C(t) = \frac{1}{5} \sum_{a,a',b=-2}^{+2} d_{0a}^{(2)}(\beta_{PN}) d_{0a'}^{(2)}(\beta_{PC}) \langle e^{ia\gamma_{PC}(0)} e^{-ia'\gamma_{PC}(t)} \rangle \times e^{i(a-a')\alpha_{CD}} d_{a,b}^{(2)}(\beta_{CD}) d_{a',b}^{(2)}(\beta_{CD}) \exp \left[-t(6D_{\perp} + b^2(D_{\parallel} - D_{\perp})) \right] \quad (12)$$

where the solid angle Ω_{DL} falls implicitly within the last diffusive term. The principal values of the diffusion tensor $D_{\parallel}$ and $D_{\perp}$ for DNA dodecamers can be measured from ^{13}C relaxation data²¹ or calculated using analytical expressions²². If the observed nuclei are aliphatic ^{13}C spins, the chemical shift anisotropy (CSA) makes only a minor contribution to relaxation, which is dominated by dipolar couplings between ^{13}C spins and their covalently bonded protons. In this case, the Zeeman relaxation rate is provided by:

$$R_{1Z}^{CH} = \frac{1}{T_{1Z}^{CH}} = N \frac{\omega_D^2}{4} (J(\omega_C - \omega_H) + 3J(\omega_C) + 6J(\omega_C + \omega_H)) \quad (13)$$

where the dipolar coupling constant is defined as $\omega_D = \frac{\mu_0}{4\pi} \frac{\hbar \gamma_C \gamma_H}{r_{CH}^3}$ and N is the number of protons coupled to the ^{13}C spin. All symbols in the coupling constant have their usual meanings. The effect of dipolar cross correlations within CH_2 groups may require the calculation of additional spectral densities; if these additional terms are appreciable, the end result may be the presence of multi-exponential relaxation²³. Cross correlation effects have been calculated by London and Avitabile²⁴ and have been found to be important for fast internal motions in the ns-ps timescale range. Slower motions considered in this work occurring at time scales longer than 1 ns do not display appreciable cross correlation effects, so equation 14 is considered a good approximation for $N=2$.

III. Methods

A) Modeling Structurally Labile Furanose Rings as Reorientational Diffusion in a Restoring Potential

In this section we derive a model that can reproduce within a single self-consistent framework the observed solid state ^2H line shape, the solid state ^2H spin-lattice relaxation rate, and the solution ^{13}C relaxation rate of a labile furanose ring in the GCGC methyltransferase DNA recognition site. In order to quantify the displacement of a C-C or C-H bond as a result of ring “puckering”, the torsional states of all five bonds of the ring must be designated. In DNA and RNA, such a complicated formulation is avoided by using the “pseudo-rotation” formalism. Like the carbon atoms in cyclopentane, the heavy atoms of furanose rings in a nucleic acid are viewed as translating along a trajectory that is roughly orthogonal to the plane of the hypothetical undistorted furanose ring. In cyclopentane, the periodic motion of the carbon atoms produces structures that would be observed if the molecule were rotating about its five-fold symmetry axis. Unlike cyclopentane, structural constraints imposed on the furanose ring from the attachment to the phosphodiester backbone and the base cause the various conformations produced by furanose “puckering” to be energetically inequivalent. Computational studies indicate that the low-energy conformations of the furanose rings in DNA and RNA cluster within two energy minima, identified by the pseudo-rotation states called C2'-endo and C3'-endo²⁵. The barrier between these low energy conformations is estimated to range from 2.1 kJ²⁶ to 6.3 kJ²⁷.

A recent solid state ^2H NMR study established that the furanose rings of the cytidine nucleotides within the GCGC moiety of the DNA dodecamer 5'-d(GATAGCGCTATC)₂ are exceptionally labile¹⁵. Instead of exchanging between conformational energy minima, (as is typical of other labile furanose rings studied by this method) these furanose rings appear to interconvert between C2' and C3'-endo conformations without traversing a measurable energy barrier. The conformational states of these rings populate a broad energy well as opposed to being distributed between two or more local minima.

Unlike the motions of a polyethylene chain, where dynamic axes are naturally defined by the C-C bonds²⁸⁻³⁰, the axis system used to describe motions of cyclic systems do not necessarily coincide with bond directions³¹. The axis system adopted here to describe the pseudo-rotational motions of furanose rings is a simple variation of a model first proposed by Herzyk and Rabczenko³². The z axis of the coordinate system used here is about 18 degrees off the direction of the C2'-C3' bond vector (Figure 1). Although the original study of Herzyk and Rabczenko³² described only displacements of the heavy atom framework, reorientation pathways of C-H bonds can be obtained by a slightly extended theory³³, where in addition to the arc-like displacements of the bonds, small twistings of the ring produce small lateral displacements as well. These small lateral displacements are neglected here, allowing the use of a simplified model where displacements of the C2'-D2'' bond is described by rotation about the z_C axis (Figure 1).

In the following, the model used to fit the ^2H line shape in the furanose rings will be briefly reviewed, and corresponding models for ^2H solid state relaxation and ^{13}C solution relaxation will be derived with the objective of determining the extent to which this model of dynamics dependent line shape also fits the solid state ^2H and solution ^{13}C relaxation data.

The Solid State ^2H Line Shape—As outlined in equations 1-4, conformational motions of the furanose ring are described as reorientational motions of bonds. In the exceptionally labile furanose rings of the *HhaI* target DNA these reorientational motions are treated in the strongly damped diffusive limit, where the diffusing 'particles' move in a restoring potential. This model is characterized by two parameters: the coefficient of internal rotational diffusion D_i , and the restoring force constant κ . The diffusive reorientation can be discretized as a sequence of short jumps between N sites, and therefore π_{ij} , which connects sites i and j will be non-zero only if $j=i\pm 1$. The discretized diffusion operator is therefore given in matrix form as³⁴:

$$\begin{aligned}\pi_{ij} &= \frac{1}{\tau} \left(\frac{W_i}{W_{i\pm 1}} \right)^{1/2}; j=i\pm 1 \\ \pi_{ij} &= \left(\pi_{i,j-1} + \pi_{i,j+1} \right); j=1 \\ \pi_{ij} &= 0, \quad \text{otherwise}\end{aligned}\tag{14}$$

where $W_i = \frac{e^{-V_i/k_B T}}{Q}$, V_i is the energy of the ith site along the reorientational trajectory and $Q = \sum_i e^{-V_i/k_B T}$.

The line shape simulations for the D2'' deuterons of nucleotides C6 and C8 (Figure 2) show that the NMR data can be well fit according to a model where the C-D2'' bonds undergo rotational diffusion in a restoring potential of the form:

$$V_i = \frac{1}{2} \kappa (\gamma_i - \gamma_0)^2\tag{15}$$

where γ_i designates the orientation of the C2'-D2'' bond at site i produced by a rotation around the z_C axis, and γ_0 is the angle associated with the minimum energy conformation (assumed to be C2'-endo). In these simulations, it is assumed that the potential seeks to restore the furanose ring to the C2'-endo conformation, which is the state of lowest energy in a B-form DNA helix. Since the pseudo-rotation coordinate system is used, the restoring force constant is not associated with the torsional motion of any single bond in the framework of the furanose ring, but rather it is proportional to the force seeking to restore the ring to the C2'-endo minimum energy conformation.

Solid State ^2H Spin-and Solution ^{13}C Spin-Lattice Relaxation—Here we derive correlation functions corresponding to the motions of the C2'-D2'' bond as modeled according to the results of the solid state deuterium line shape studies¹⁵. Rotational diffusion in restoring potentials has been primarily modeled in the magnetic resonance literature using potentials of the following form³⁵:

$$V(\gamma) = \lambda(1 - \cos^n \gamma) \quad (16)$$

The Maier-Saupe case (corresponding to $n=2$), has been extensively examined by Nordio and coworkers³⁶ and Freed³⁷ for the case of reorientational motion of molecules in liquid crystalline phases. In the remainder, we assume that the relevant motions can be described by a potential of the form given in equation 15.

The experimental line shape data strongly indicate that the dynamics of the C2'-D2'' bond is that of a one-dimensional, Brownian diffuser. In this case, the probability of a bond being distributed at γ at time t is obtained by solving the diffusion equation

$$\begin{aligned} \frac{\partial P}{\partial t} &= D \frac{\partial^2 P(\gamma, t)}{\partial \gamma^2} + \frac{D}{k_B T} \frac{\partial}{\partial \gamma} \left(\frac{dV(\gamma)}{d\gamma} P(\gamma, t) \right) \\ &= D \frac{\partial^2 P(\gamma, t)}{\partial \gamma^2} + \frac{\kappa D}{k_B T} \frac{\partial}{\partial \gamma} (\gamma P(\gamma, t)) \end{aligned} \quad (17)$$

Given the initial condition, $P(\gamma, 0) = \delta(\gamma - \gamma_0)$, equation 17 has the form of an Ornstein-Uhlenbeck process with the well-known solution³⁸

$$P(\gamma, t | \gamma_0) = \left(\frac{\kappa}{2\pi k_B T (1 - e^{-2\kappa B_i t})} \right)^{1/2} \times \exp \left[-\frac{\kappa(\gamma - \gamma_0 e^{-\kappa B_i t})^2}{2k_B T (1 - e^{-2\kappa B_i t})} \right] \quad (18)$$

where the mobility B_i is related to the internal diffusion coefficient by $B_i k_B T = D_i$.

If we assume the only motion affecting the Zeeman relaxation of the D2'' deuteron is the libration of the furanose ring, as specified by equations 17 and 18, the correlation function in equations 5 and 12 is given by:

$$\begin{aligned} \Gamma_{a, a'}(t) &= \langle e^{ia\gamma_0} e^{-ia'\gamma} \rangle = \int_{-\pi}^{+\pi} d\gamma \int_{-\pi}^{+\pi} d\gamma_0 e^{-ia\gamma_0} e^{ia'\gamma} P(\gamma, t | \gamma_0) W(\gamma_0) \\ &= \left(\frac{\kappa}{2\pi k_B T} \right)^{1/2} \left(\frac{\kappa}{2\pi k_B T (1 - e^{-2\kappa B_i t})} \right)^{1/2} \int_{-\pi}^{+\pi} d\gamma \int_{-\pi}^{+\pi} d\gamma_0 e^{-ia\gamma_0} e^{ia'\gamma} \times \exp \left[-\frac{\kappa\gamma_0^2}{2k_B T} \right] \times \exp \left[-\frac{\kappa(\gamma - \gamma_0 e^{-\kappa B_i t})^2}{2k_B T (1 - e^{-2\kappa B_i t})} \right] \end{aligned} \quad (19)$$

where the *a priori* probability, $W(\gamma_0)$ is:

$$W(\gamma_0) = \left(\frac{\kappa}{2\pi k_B T} \right)^{1/2} e^{-\kappa \gamma_0^2 / 2k_B T} \quad (20)$$

The integrals in equation 19 can be performed numerically. Alternatively, with the following,

substitutions: $z = \frac{e^{-\kappa B t}}{2}$, $x = \left(\frac{\kappa}{2k_B T} \right)^{1/2} \gamma = \beta^{1/2} \gamma$, and $x_0 = \beta^{1/2} \gamma_0$, Equation 18 acquires the form:

$$P(x, t | x_0) = \left(\frac{\beta}{\pi} \right)^{1/2} e^{-x^2} \left(\frac{1}{1 - 4z^2} \right)^{1/2} \exp \left[\frac{4z}{1 - 4z^2} (x_0 x - z x^2 - z x_0^2) \right] \quad (21)$$

The expression in equation 21 is proportional to series of products of Hermite polynomials, as given by Mehler's expansion^{39, 40}

$$\left(\frac{1}{1 - 4z^2} \right)^{1/2} \exp \left[\frac{4z}{1 - 4z^2} (x_0 x - z x^2 - z x_0^2) \right] = \sum_{n=0}^{\infty} \frac{Z^n}{n!} H_n(x_0) H_n(x) \quad (22)$$

where $H_n(x) = (-1)^n e^{x^2} \frac{\partial^n}{\partial x^n} e^{-x^2}$. Using equation 22, equation 19 becomes

$$\begin{aligned} \Gamma_{a,a'}(t) \langle e^{-ia'\gamma_0} e^{ia'\gamma} \rangle &= \int_{-\pi}^{+\pi} d\gamma \int_{-\pi}^{+\pi} d\gamma_0 e^{-ia\gamma_0} e^{-ia'\gamma_t} P(\gamma, t | \gamma_0) W(\gamma_0) \\ &= \frac{\beta}{\pi} \sum_{n=0}^{\infty} \frac{Z^n}{n!} \times \int_{-\pi}^{+\pi} d\gamma e^{ia'\gamma} e^{-\beta\gamma^2} H_n(\beta^{1/2}\gamma) \int_{-\pi}^{+\pi} d\gamma_0 e^{-ia\gamma_0} e^{-\beta\gamma_0^2} H_n(\beta^{1/2}\gamma_0) \\ &= \frac{\beta}{\pi} \sum_{n=0}^{\infty} \frac{Z^n}{n!} \times I_{a'}^{(n)} \times I_{-a}^{(n)} = \sum_{n=0}^{\infty} A_{a,a'}^{(n)} e^{-t/\tau_n} \end{aligned} \quad (23)$$

where

$$A_{a,a'}^{(n)} = \frac{\beta}{\pi} \frac{I_{a'}^{(n)} I_{-a}^{(n)}}{2^n n!}; \quad I_a^{(n)} = \int_{-\pi}^{+\pi} d\gamma e^{ia\gamma} e^{-\beta\gamma^2} H_n(\beta^{1/2}\gamma); \quad \frac{1}{\tau_n} = n\kappa B_i = n\kappa B_i = \frac{n\kappa D_i}{k_B T} \quad (24)$$

In contrast to equation 19, which expresses $\Gamma_{a,a'}(t)$ in terms of an integral of a superexponential, equation 23 expresses $\Gamma_{a,a'}(t)$ as an infinite series of products of integrals of Hermite

polynomials. The amplitudes $A_{a,a'}^{(n)}$ vary as $\frac{1}{2^n n!}$, and as Table 1 shows, the series converges for values of the restoring force constant κ relevant to the present study, within the first 4 to 5 terms. If the only motion affecting the Zeeman relaxation rate R_I is diffusion in a restoring potential, the powder-averaged correlation function in the solid state is then:

$$\bar{C}(t) = \frac{1}{5} \sum_{a=-2}^{+2} d_{0a}^{(2)}(\beta_{pc}) d_{0a}^{(2)}(\beta_{pc}) \Gamma_{aa}(t) = \frac{1}{5} \sum_{n=0}^{\infty} \sum_{a=-2}^{+2} d_{0a}^{(2)}(\beta_{pc}) d_{0a}^{(2)}(\beta_{pc}) A_{a,a}^{(n)} e^{-t/\tau_n} \quad (25)$$

where $A_{a,a}^{(n)}$ is given by equation 24. The relevant powder-averaged spectral density is:

$$\begin{aligned}
 J(\omega) &= \frac{2}{5} \sum_{n=0}^{\infty} \sum_{a=-2}^{+2} d_{0a}^{(2)}(\beta_{pc}) d_{0a}^{(2)}(\beta_{pc}) A_{a,a}^{(n)} \int_0^{+\infty} dt e^{-t/\tau_n} \cos \omega t \\
 &= \frac{2}{5} \sum_{n=0}^{\infty} \sum_{a=-2}^{+2} d_{0a}^{(2)}(\beta_{pc}) d_{0a}^{(2)}(\beta_{pc}) A_{a,a}^{(n)} \frac{\tau_n}{1+\omega^2 \tau_n^2}
 \end{aligned} \quad (26)$$

The analogous solution NMR spectral density (assuming rigid cylindrical tumbling) is:

$$\begin{aligned}
 J(\omega) &= \frac{2}{5} \sum_{n=0}^{\infty} \sum_{a,a',b=-2}^{+2} d_{0a}^{(2)}(\beta_{p1}) d_{0a'}^{(2)}(\beta_{p1}) A_{a,a'}^{(n)} e^{i(a-a')\alpha_{1,D}} d_{a,b}^{(2)}(\beta_{1,D}) d_{a',b}^{(2)}(\beta_{1,D}) \\
 &\times \int_0^{+\infty} dt \exp \left[-t \left(6D_{\perp} + b^2 (D_{\parallel} - D_{\perp}) + \tau_n^{-1} \right) \right] \cos \omega t \\
 &= \frac{2}{5} \sum_{n=0}^{\infty} \sum_{a,a',b=-2}^{+2} d_{0a}^{(2)}(\beta_{p1}) d_{0a'}^{(2)}(\beta_{p1}) e^{i(a-a')\alpha_{1,D}} d_{a,b}^{(2)}(\beta_{1,D}) d_{a',b}^{(2)}(\beta_{1,D}) A_{a,a'}^{(n)} \frac{\lambda_{n,b}}{1+\omega^2 \lambda_{n,b}^2}
 \end{aligned} \quad (27)$$

$$\text{where } \lambda_{n,b} = \left(6D_{\perp} + b^2 (D_{\parallel} - D_{\perp}) + \tau_n^{-1} \right)^{-1}$$

To model the relaxation of ^2H nuclei in DNA in the hydrated solid state, equation 26 is substituted into equation 10. To model the solution dipolar relaxation of aliphatic ^{13}C nuclei in DNA, equation 27 is substituted into equation 13.

B) Sample preparation

Synthesis of selectively furanose-deuterated nucleosides and subsequent preparation of selectively deuterated phosphoramidites has been described previously¹⁵. A uniformly labeled $^{13}\text{C}/^{15}\text{N}$ non-palindromic dsDNA dodecamer with the sequence [5'-(dGGTAGCGCTATT)-3']-[5'-(dAATAGCGCTACC)-3'] was purchased from *BioQuantis*, as described earlier⁴¹.

C) NMR experiments

Solid state ^2H NMR spin-lattice relaxation times were recorded using an inversion recovery pulse sequence, which incorporated a 180° composite pulse to ensure broadband excitation.

⁴² In order to obtain powder-averaged Zeeman spin-lattice relaxation times, T_1^{-Q} , the integrated intensity of the powder spectrum was monitored as a function of recovery time and analyzed using a non-linear least squares fitting routine⁴³. Solid state NMR spectra and relaxation rates were obtained as described in Meints et al³³ at a magnetic field of 11.7T, corresponding to a deuterium Larmor frequency of 76.8 MHz. The ^{13}C solution T_1 values were obtained as described by Shajani & Varani⁴¹. ^{13}C Relaxation rates were recorded at 11.7T corresponding to a Larmor frequency of 125.0 MHz.

D) Line shape analysis

In order to simulate the ^2H line shape a form for the matrix $A=i\omega+\pi$ is required. The diagonal matrix ω , whose form is given in equation 4, is evaluated given a description of the trajectory of the C-D bond. This trajectory is determined from an extension of the theory by Herzyk and Rabczenko³² and the exchange matrix π is defined as in equation 14. In order to construct this matrix, we required values for the potential V and the internal diffusion coefficient D_i .

The ^2H line shapes for the furanose ring deuterons of nucleotides C6 and C8 were best fit using

a simple restoring potential $V(\gamma) = \frac{K}{2}(\gamma - \gamma_0)^2$, where γ is referenced to the coordinate system shown in Figure 1. Solid state ^2H line shapes were evaluated in the context of this model with the program MXET1¹⁵. For the ^2H line shape simulations it was assumed that

$QCC_{static} = e^2 q Q / h = 170 \text{ kHz}$, and that the principal axis of the (axial) EFG tensor is collinear with the C-D bond axis.

E) Simulation of the Relaxation Rates

Both solid and solution spin-lattice relaxation rates were analytically simulated in Mathematica 6.0, using the dynamic model described in section III to predict both experimental solid state powder-averaged ^2H and solution ^{13}C spin-lattice relaxation times. Solid state ^2H relaxation rates and ^{13}C relaxation rates were calculated for the motion of the C2'-D2'', C2'-H2'' and C1'-H1' bonds of nucleotides C6 and C8 only.

For the solution calculations, it was assumed that the spin-lattice relaxation for $^{13}\text{C}2'$ is dominated by the dipolar coupling to the covalently bound protons. The DNA dodecamer was assumed to tumble as a rigid cylinder and the vibrationally averaged C-H bond length was assumed to be $1.10 \text{ \AA}$. Elements of the axially symmetric diffusion tensor were obtained using

the standard formulas²¹ $D_{\perp} = \frac{k_B T}{f_{\perp}}$ and $D_{\parallel} = \frac{k_B T}{f_{\parallel}}$. The two rotational friction coefficients were calculated using the expressions:

$$f_{\parallel} = f_0 \times 0.64 \times \left(1 + \frac{0.677}{P} - \frac{0.183}{P^2} \right) \quad (28)$$

$$f_{\perp} = f_0 \frac{2P^2}{9(\ln P + \delta)} \quad (29)$$

where $f_0 = 8\pi\eta R_e^3$, and the viscosity $\eta = 0.89 \times 10^{-3} \text{ kg m}^{-1} \text{ s}^{-1} = 0.891 \text{ cP}$ at $T = 300 \text{ K}$.

$R_e = \left(\frac{3}{2P^2} \right)^{1/3} \left(\frac{L}{2} \right)$ is the radius of the sphere of equal volume to a cylindrical DNA for which $P = L/2b$. For a DNA dodecamer the length $L = 38 \text{ \AA}$, the hydrodynamic radius $b = 10 \text{ \AA}$, so $P = 1.9$. The end cap correction in equation 29 is, according to Tirado and Garcia **del la Torre**²²

$$\delta = -0.662 + \frac{0.917}{P} - \frac{0.050}{P^2} \quad (30)$$

With these assumptions, and using equations 29-30, $D_{\perp} = 3.59 \times 10^7 \text{ s}^{-1}$ and $D_{\parallel} = 7.68 \times 10^7 \text{ s}^{-1}$,

with the ratio $\frac{D_{\parallel}}{D_{\perp}} \approx 2.1$.

As indicated by equation 27, in the calculation of solution relaxation rates it is necessary to relate the axis system within which the local motion is described, to the axis system associated with the rotational diffusion of the dodecamer. In the case of a DNA dodecamer, assumed to be tumbling as a rigid cylinder, the latter axis system is defined as the principal axis system of the diffusion tensor, where the element $D_{\parallel}$ is assumed to exist parallel to the helix axis. At minimum is required the angle between dynamic axis of the harmonically moving C-D bond and the helical axis, which in equation 27 is designated $\gamma_{1,D}$. Because of local structural variability in DNA, this angle may vary. Results for the solution simulations are therefore plotted as a function of the angle $\beta_{1,D}$ (designated simply as β in Figure 8) to show their dependence on it. The interior angle of the C-H bond vector to the plane of the interior motion was taken either to be 88.5° (for C6-C2' and C8-C2') or 127.5° (for C6-C1' and C8-C1'). The

solution calculations shown do not include contributions from chemical shift anisotropies; their effects on relaxation times were also simulated but for aliphatic carbons with anisotropy of about 40 ppm, they exhibit less than 3% effect on relaxation times²¹.

IV. Results

In order to describe the line shapes observed for C6 and C8 and plotted in Figure 2, it is necessary to take into account other types of motion that are present in the sample besides the local puckering of the furanose rings. Collective long range bending and torsional motions can be neglected for a dodecameric DNAs at the hydration levels we used ($W=10$)^{14,15}. Uniform end-to-end tumbling can also be neglected (although not in solution), since this type of motion is restricted in the solid-state, even with a sample of intermediate hydration ($10 < W < 20$). Previous work has shown that restricted uniform rotation of the DNA around the helix axis occurs at $W=10$ and above, and can be effectively simulated by a six-site jump,²⁸ with a half angle of $\theta = 20^\circ$ (representing the orientation of the local dynamic axis of the C2'-D bond with respect to the longitudinal helix axis) and values of $\Phi = 0^\circ, 60^\circ, 120^\circ, 180^\circ, 240^\circ$, and 300° for the six sites; a rate constant of $k \approx 1.0 \times 10^4$ Hz was successfully used to model these motions. The use of these parameters for the overall helix motion has produced good agreement in previous work for several different DNA samples with different types of local motions.¹⁴

The simulated spectra that could fit the experimental results of Figure 2 were generated by convolution of the uniform helical rotation and the local motion of the furanose ring. The local motion was simulated as described in equations 1-4, where an exchange matrix is constructed with equations 14 and 15. Numerical evaluation with MXET1 obtained a best fit for $D_i = 1.8 \times 10^7 \text{ rad}^2 \text{ s}^{-1}$, and $\kappa = 5 \text{ k}_B T$, where $T = 300 \text{ K}$, corresponding to about $12.5 \text{ kJ rad}^{-2} \text{ mol}^{-1}$. Minor differences in the powder line shape of C6 and C8 are likely due to small differences in the hydration, while slightly different levels of HDO contribute to different levels of intensity at the spectral centers. Differential hydration may also cause small differences in the rates of the slow helical motions.

The powder-averaged spin-lattice recovery curves for the furanose deuterons of nucleotides C6 and C8 are shown in Figure 3. Best fits to the recovery curves yield powder-averaged ^2H T_1 's of $29 \pm 3 \text{ ms}$ for the furanose deuteron of nucleotide C6 and $22 \pm 4 \text{ ms}$ nucleotide C8 (Table 3); these values are statistically indistinguishable from one another. Of course, a powder-average reflects a sum over all crystallites so a powder-averaged recovery of longitudinal magnetization should in principle be non-exponential. But as discussed in Vold & Vold¹⁸ and reference cited therein, in practice deviations from exponential recovery are usually small, especially in the intermediate exchange regime.

A) Predicting Deuterium Relaxation Times from Static Deuterium Line shapes

Having established the characteristics of the furanose motions from the simulation of the static deuterium line shapes, we then applied the same formalism to calculating solid state and then solution relaxation times. Calculated T_1 values for both solution and solid state are shown in Figure 4 for a range of internal diffusion constants. We found as expected that the solution measurements are quite sensitive to motions above the ns level and quite insensitive to motions of ms and slower. In the intermediate region of the Larmor frequency, Figure 4b, both solution and solid state times depend on the internal diffusion constant, or in other words on the details of the internal motion modeled, though the effect is more dramatic in the solid state where there is an absence of global reorientational motion. Simulated powder-average deuterium spin-lattice relaxation times are plotted in Figure 5 as functions of internal diffusion coefficient

D_i and the ratio $\frac{\kappa}{2k_B T}$, which were obtained from equation 10 using the powder-averaged spectral density given in equation 26. The helix motion was neglected in the calculation of

the ^2H T_1 , since it occurs at a very low rate and therefore does not affect the ^2H spin-lattice relaxation to any measurable extent.

These plots demonstrate that the ^2H spin-lattice relaxation rates are strongly dependent on D_i but do not vary significantly as a function of the restoring force constant for values of $\frac{\kappa}{2k_B T}$ less than 2.0 and more than 0.4. This relatively uniform behavior in κ is simply because the observed range of κ values occurs near the minimum in T_1 ; however, when $\frac{\kappa}{2k_B T} > 2$, T_1 increases rapidly with κ (Figures 4a and 4b).

When we attempted to simulate the experimentally observed relaxation times using the values of κ and D_i derived from our line shape simulations, we obtained T_1 of 26 ms (Table 2). This result is within experimental error of our results for both C6 and C8. These simulations also indicate that a small difference in D_i , (which cannot be discerned easily in the line shape simulation) could easily account for the small differences in the observed T_1 values between these two nucleotides.

B) Predicting Solution ^{13}C Relaxation Times from Static Deuterium Line shapes

Next we attempted to calculate the solution ^{13}C relaxation rates using the model of local motion derived from the solid state powder lineshape and further supported by solid state ^2H NMR relaxation rates. In order to calculate solution relaxation times using the same formalism, the global rotational diffusion of the DNA had to be described as well, since these motions have a major contribution to solution relaxation. Thus, we assumed that global reorientational motion could be described by an ideal B-form DNA dodecamer with a ratio of hydrodynamic

parameters given by $\frac{D_{\parallel}}{D_{\perp}} \approx 2.1$, as derived from the shape of the DNA helix and described above. The simulations of the solution $^{13}\text{C}2'$ spin lattice relaxation time (using equations 13 and 27) demonstrate how the relaxation times depend on D_i , κ , and $D_{\perp}$ (Figures 6 and 7). The ^{13}C spin-relaxation time is only moderately sensitive to changes in D_i and κ , whereas even modest variation in $D_{\perp}$ leads to large changes. Although the dependence of T_1 on D_i increases as the value of $D_{\perp}$ diminishes toward $10^6 \text{ rad}^2 \text{ s}^{-1}$, T_1 varies only slightly as a function of D_i for values where $D_{\perp}$ is close to or greater than the Tirado-Garcia del la Torre²² theoretical value of $3.65 \times 10^7 \text{ rad}^2 \text{ s}^{-1}$. The variation of T_1 with $D_{\perp}$ is shown on a logarithmic scale (Figure 6b) to emphasize the relative sensitivity of T_1 to D_i and $D_{\perp}$. The sensitivity of T_1 with respect to κ and $D_{\perp}$, is shown also on a logarithmic scale in Figure 7, where $D_i = 1.8 \times 10^7 \text{ rad}^2 \text{ s}^{-1}$, the value derived from the ^2H solid state line shape. When κ and $D_{\perp}$ are varied together over similar ranges (by up to a factor of 10-15), the resulting variation in T_1 is much greater for $D_{\perp}$ than for κ . In contrast, there are substantial variations in T_1 as a function of the orientation between the principal axis of the diffusion tensor z_D and the z axis of the local dynamics frame, z_C , as quantified by the angle $\beta_{1,D}$, (designated simply β in Figure 7).

Small variations along the sequence were reported for the solution ^{13}C T_1 's for the $^{13}\text{C}2'$ spins of this 12-mer DNA¹⁷. The simulations presented in Figure 6-8 strongly suggest that the most likely source of these small differences between for example the C6 and C8 nucleotide is local structural variations that cause the angle β to vary between the two nucleotides.

In order to calculate the $^{13}\text{C}2'$ spin lattice relaxation times for the present DNA dodecamer,

we used the values of $\frac{\kappa}{k_B T}$ and D_i obtained by fitting the solid state ^2H line shapes (2.5 and $1.8 \times 10^7 \text{ rad}^2 \text{ s}^{-1}$, respectively) and values of $\frac{D_{\parallel}}{D_{\perp}} = 2.1$ and $D_{\perp} = 3.65 \times 10^7 \text{ rad}^2 \text{ s}^{-1}$ that were obtained by describing global rotational motion of the DNA according to the approach of Tirado

and Garcia del la Torre²². The calculated $^{13}\text{C}'$ spin lattice relaxation time of 230 ms compared very well with the observed experimental values of 240.5 ± 14.4 ms for $^{13}\text{C}'$ in nucleotide C6 and 202.9 ± 12.2 ms for $^{13}\text{C}'$ in nucleotide. Using equations 13 and 27, and again assuming $\beta = -40$ degrees, we can also calculate the spin lattice relaxation times for the $^{13}\text{C}'$ spins of nucleotides C6 and C8. The simulated values (436 ms) compare again very well to the experimental values (447.6 ± 4.5 ms and 449.7 ± 4.5 ms, Table 2).

V. Discussion

Solution NMR has been widely used to probe biomolecular dynamics in proteins and, less often, in DNA and RNA¹⁰⁻¹². This approach is a very powerful tool to investigate motions in the ns-ps and ms- μ s regimes, but the intervening broadly defined μ s-ns range is less penetrable to most solution relaxation measurements. Thus, motions that may occur on this time scale have for the most part been neglected in solution NMR studies of biomolecular dynamics. However, solid state NMR line shape and relaxation studies in both proteins and nucleic acids have long reported that this intermediate time scale is host to a multitude of motions. The disparate sensitivities of these two techniques to motions detected by solution relaxation techniques have been recently discussed for both DNA¹⁷ and RNA⁴⁴. For example, for the furanose ring of the *HhaI* methyltransferase target DNA, the solid state ^2H T_1 's of C8 and A10 differ by almost 400% while the solution ^{13}C T_1 's at the same sites in the same molecules vary by less than 10%. A few recent solution NMR studies of both RNA and proteins based on residual dipolar couplings have confirmed the motional richness of this regime, by contrasting order parameter values obtained by relaxation measurements and order parameters derived from residual dipolar couplings.^{45, 46} Thus, relying exclusively on solution relaxation measurements runs the risk that motions within this time scale will go undetected, and an incomplete or incorrect description of dynamics will be obtained.

We are confident that the joint application of solution and solid state NMR methods to studies of dynamics has the potential to overcome this limitation of solution NMR methods to studying dynamics. However, methods are needed in order to merge the experimental results obtained by these two methods in a common interpretative framework. In this paper, we introduced a theoretical formalism that describes at the same time solution relaxation data and solid state NMR line shape and relaxation data for the particular case of furanose rings in DNA. Although the specific models used to fit solution and solid state relaxation data will differ, the important point of this work is that a far more complete view of the internal motions in biological molecules is obtained by analyzing data obtained by at least two dynamic spectroscopies, whose sensitivities to dynamic time scales are complementary, through a common theoretical framework.

We demonstrate (Table 2) that a common model of dynamics that describes the internal motions of the furanose rings derived from the analysis of solid state ^2H measurements accurately predicts solid state and solution relaxation times. This was achieved by fitting the solid state line shapes to specific motional models and by describing global rotational diffusions of the DNA using well-established hydrodynamic models. Thus, the motions observed in the hydrated solid DNA together with the overall motions of the molecule in solution are sufficient to account for the solution spin-lattice relaxation and can be described through a common formalism.

We emphasize that this approach is orthogonal to the commonly used model-free analysis^{47, 48} of solution relaxation times in that a specific dynamic model, obtained from a partially averaged solid state NMR powder pattern, is used as a starting point for analyzing solution relaxation data. Far from neglecting the detailed atomic motions responsible for the observed experimental results, our approach is explicitly model-dependent and therefore provides

information on the detailed nature of the localized biomolecular motions. The results demonstrate that the very large differences we have reported experimentally in solid state relaxation times and line shapes along the sequence of the *HhaI* target DNA (Table 3), as compared to small differences in solution relaxation times, are due to local motions in the DNA furanose rings that occur in the μ s-ns timescale, a time scale to which solid state NMR is strongly sensitive and to which solution NMR is less sensitive.

VI. Conclusion

We have demonstrated here an approach for describing biomolecular motions over a wide dynamic range using solution and solid state relaxation and solid state line shape experimental results analyzed through a common formalism. The model dependence of the approach provides insight into the detailed atomic characteristics of the motions responsible for the experimental observables. The present results emphasize that by bridging the gap between ns and μ s it is possible to obtain a more complete description of biological motion. We envision a protocol to investigate dynamics in biology where ^2H solid state NMR and ^{13}C solution NMR are both used to probe motion within the ps-ns time scale; solid state ^2H NMR is used to probe the ns- μ s time scale; and relaxation dispersion experiments in solution and solid-state ^2H line shape analysis are used to probe the ms- μ s regime.

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a

Figure 1.

a: Coordinate systems fixed to the local nucleotide structure used in the calculation of ^2H solid state NMR $T_{1\rho}$'s and ^{13}C solution NMR T_1 's. Z_D represents the z axis of the principal axis system of the electric field gradient (EFG) tensor, and is parallel to the C-D bond axis. The coordinate system to which the local dynamics of the furanose ring is referenced has its z axis (Z_C) about 18 degrees off the C2'-C3' bond axis.

b: An additional coordinate system is used for the purpose of calculating the ^{13}C solution T_1 . The z axis Z_D of the uniaxial diffusion tensor is assumed to be parallel to the helix axis. The ^{13}C T_1 is calculated as a function of the angle between the Z_C and Z_D axes.

Figure 2.

Experimental powder pattern line shapes and corresponding simulations recorded for the D2' deuterons of nucleotides C6 (a) and C8 (b). The data were collected at hydration levels of $W=10$ and temperature $T=300\text{K}$. The line shape simulations are based on a model described in the text, where dynamics of the furanose rings is treated as diffusion in a potential that seeks to restore the furanose ring to the C2' endo conformation.

Figure 3.

Inversion-recovery curves for the D2⁺ deuterons of nucleotides C6 and C8, at hydration levels W=10 of ten water molecules per nucleotide and T=300K. Curve fitting results in (a) $T_1=29 \pm 3$ ms for C6 and (b) $T_1=22 \pm 4$ ms for C8.

Figure 4.

Calculated T_1 relaxation times for both solution and solid NMR as a function of internal diffusion, varying from diffusion speeds well below the Larmor (a) frequency to ones significantly above (c). The global reorientational motion, determined by $D_{\perp}$, and barrier height $\kappa/2kT$ were fixed to values determined to fit experimental line shape data for the motions of cytidine furanose rings.

Figure 5.

Powder averaged ^2H solid state T_1 relaxation times calculated using equations 10 and 30 and

displayed as a function of the internal diffusion coefficient D_i for values of $\frac{\kappa}{2k_B T}$ between 0

and 5. The same functions are displayed over a wider range of $\frac{\kappa}{2k_B T}$ values. The term ω_D refers to a value of D_i equal to the deuterium Larmor frequency $\omega_D = 2\pi\nu_D$.

Figure 6.

Solution-state $^{13}\text{C}2'$ T_1 relaxation times calculated using equations 13 and 31, and plotted as a function of D_i and $D_{\perp}$. These results were obtained assuming that the global reorientational motion is describable by an ideal B-form DNA dodecamer with a ratio of hydrodynamic

diffusion parameters $\frac{D_{\parallel}}{D_{\perp}} \approx 2.1$. Because the furanose CSA's are relatively small, pure dipolar relaxation was assumed, and cross correlation effects were neglected.

Figure 7. Solution T_1 relaxation times for $^{13}\text{C}2'$ calculated using equations 13 and 31, and plotted as a function of $\frac{\kappa}{2k_B T}$ and $D_{\perp}$. The same assumptions were made as in Figure 6.

Figure 8.

Solution T_1 for $^{13}\text{C}2'$ nuclei calculated using equations 13 and 31, and plotted as a function of β and D_i . For these calculations, $D_{\perp} = 3.65 \times 10^7 \text{ rad}^2 \text{ s}^{-1}$ and $\frac{D_{\parallel}}{D_{\perp}} = 2.1$ function of β and D_i . For these calculations, $D_{\perp} = 3.65 \times 10^7 \text{ rad}^2 \text{ s}^{-1}$ and $\frac{D_{\parallel}}{D_{\perp}} = 2.1$.

Table 1

Amplitudes $A_{aa'}^{(n)}$ calculated for $n=0-8$ and $\frac{\kappa}{2k_B T}=2.5$

n	$A_{00}^{(n)}$	$A_{11}^{(n)}$	$A_{22}^{(n)}$	$A_{21}^{(n)}$
0	1	0.819	0.449	0.607
1	0	0.164	0.359	0.243
2	0	.0164	0.144	0.049
3	0	1.09×10^{-3}	0.0383	6.47×10^{-3}
4	0	5.46×10^{-5}	7.67×10^{-3}	6.47×10^{-4}
5	0	2.18×10^{-6}	1.23×10^{-3}	5.18×10^{-5}
6	0	7.28×10^{-8}	1.64×10^{-4}	3.45×10^{-6}
7	0	2.08×10^{-9}	1.87×10^{-5}	1.97×10^{-7}
8	0	0	1.87×10^{-6}	9.91×10^{-9}

Experimental and simulated spin lattice relaxation times obtained for the furanose deuterons and ^{13}C spins in nucleotides C6 and C8

Table 2

	Location in the sequence	Experimental results (ms)	Theoretical predictions (ms)	Angle with helical axis	Angle with internal motional axis
T_1 solid	C8-C2'-D	22 ± 4	26	N/A	88.5°
T_1 solid	C6-C2'-D	29 ± 3	26	N/A	88.5°
T_1 solution	C8-C2'-H2"	202.9 ± 12.2	230	-40°	88.5°
T_1 solution	C6-C2'-H2"	240.5 ± 14.4	230	-40°	88.5°
T_1 solution	C8-C1'-H"	449.7 ± 4.5	436	-40°	109.5°
T_1 solution	C6-C1'-H"	447.6 ± 4.5	436	-40°	109.5°

Table 3

Comparison of solid state NMR spin lattice relaxation times for the furanose deuterons in and adjacent to the GCGC recognition site with ^{13}C solution NMR spin-lattice relaxation times

Label Site	^2H solid state T_1 (ms) ^a	^{13}C solution T_1 (ms) ^b	^{13}C solution T_1 (ms) ^c
A4	65 ± 5	210.4±16.5	424.4±6
G5	59 ± 4	198.6±14	424.4±6
C6	29 ± 3	240.5±14.4	449.7±4.5
G7	69 ± 5	244.8±19	418.2±4
C8	22 ± 4	202.9±12.2	447.6±4.5
A10	82 ± 7	216.6±13	384±4.5

^aD₂''^b $^{13}\text{C}_{2'}$ ^c $^{13}\text{C}_{1'}$