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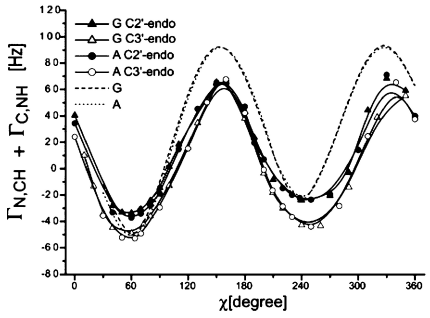

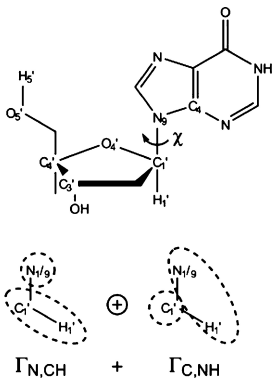

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Sugar Pucker Modulates the Cross-Correlated Relaxation Rates across the Glycosidic Bond in DNA

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Abstract: The dependence of N1/9 and C1′ chemical shielding (CS) tensors on the glycosidic bond orientation (χ) and sugar pucker (P) in the DNA nucleosides 2′-deoxyadenosine, 2′-deoxyguanosine, 2′-deoxycytidine, and 2′-deoxythymidine was studied using the calculation methods of quantum chemistry. The results indicate that these CS-tensors exhibit a significant degree of conformational dependence on χ and P structural parameters. The presented data test underlying assumptions of currently established methods for interpretation of cross-correlated relaxation rates between the N1/9 chemical shielding tensor and C1′−H1′ dipole−dipole (Ravindranathan et al. *J. Biomol. NMR* **2003**, *27*, 365−75. Duchardt et al. *J. Am. Chem. Soc.* **2004**, *126*, 1962−70) and highlight possible limitations of these methods when applied to DNA.

Introduction

The cross-correlated relaxation of double- and zero-quantum coherences has been introduced into high-resolution NMR only recently to extract structural information from biomolecules.[1] The cross-correlated relaxation rates (Γ) have widespread applications in the determination of structural parameters such as torsion angles in proteins,[2] conformation of O-glycosidic linkage in carbohydrates,[3,4] or characterization of sugar puckers,[5] phosphodiester backbone,[6] and hydrogen-bond length in nucleic acids.[7] Two newly developed methods exploiting the cross-correlated relaxation rates between the C1′−H1′ dipole−dipole and N1/9 chemical shielding (CS) tensor ($\Gamma_{N1/9,C1'H1'}$) have been established as a source of information on structure and dynamics around the glycosidic linkage in ribonucleic acids (RNAs).[8,9] In the framework of Duchardt's method,[9] the modulation in $\Gamma_{N1/9,C1'H1'}$ is expressed as a simple geometric term relating the orientation between the principal axis of the N1/9 chemical shielding tensor and the C1′−H1′ vector to the glycosidic torsion angle χ. In contrast to this approach, Ravindranathan's method[8] uses $\Gamma_{H1/9,C1'H1'}$ and a priori known structural information to assess the information about local and overall dynamics. The method is based on comparison between experimentally determined $\Gamma_{N1/9,C1'H1'}$ and $\Gamma_{N1/9,C6/8H6/8}$.[8] In the case when C1′−H1′ interacts with the N1/9 CS-tensor, interacting spins are located in different structural moieties of a nucleoside and $\Gamma_{N1/9,C1'H1'}$ corresponds not only to overall molecular tumbling but also to the internal motion around the glycosidic bond. In the case of the N1/9 CS-tensor interacting with C6/8−H6/8 dipole−dipole ($\Gamma_{N1/9,C6/8H6/8}$), both the tensor and the dipolar vector are located within the nucleic acid base and thus are rigidly fixed with respect to each other. In this case, the cross-correlated relaxation rates reflect only overall molecular tumbling.

Ravindranathan's and Duchardt's methods are valuable tools for characterization of nucleic acid structure and dynamics. In these methods, the interpretation of $\Gamma_{N1/9,C1'H1'}$ relies on the following assumptions: (a) the magnitude and orientation of the N1/9 and C1′ CS-tensors are known and (b) N1/9 and C1′

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CS-tensors are independent of the local structure of the nucleoside. While both experimental[10,11] and theoretical data[10,12,13] on N1/9 and C1′ CS-tensors in ribo- and 2′-deoxyribonucleosides are available in the literature, very little is known about their dependence on local nucleic acid structure. There are some established correlations of C1′ isotropic chemical shift with sugar pucker and glycosidic torsion in nucleic acids.[14] However, much less information is available about the anisotropy of the C1′ CS-tensor. Dejaegere and Case reported that chemical shielding anisotropy (CSA) at C1′ in 2′-deoxythymidine is sensitive to the sugar pucker.[12] However, nothing is known about the dependence of the C1′ CSA on the orientation of glycosidic torsion, and no information about the dependence of the N1/9 CSA on either the sugar pucker or glycosidic torsion was obtained so far.

In this work, quantum chemical calculation methods are used to investigate how the N1/9 and C1′ chemical shielding tensors depend on the orientation of the glycosidic torsion angle and sugar pucker in 2′-deoxynucleosides to show the applicability of Ravindranathan's and Duchardt's methods to deoxyribonucleic acids (DNAs).

Theoretical Background

In the case of a molecule undergoing isotropic rotational diffusion and neglecting internal motions, the expression for the cross-correlated relaxation rates $\Gamma_{N1/9,C1'H1'}$ between the N1/9 chemical shielding tensor and the C1′−H1′ dipole−dipole vector is given by[8]

$$\Gamma_{N1/9,C1'H1'} = \frac{2}{3}\left(\frac{\mu_0\hbar}{4\pi}\right)\frac{\gamma_H\gamma_C}{r_{C1'H1'}{}^3}\gamma_N B_0 \sigma_{N1/9,C1'H1'}\sum_q J(\omega_q) \quad (1)$$

$$\sigma_{N1/9,C1'H1'} = \sum_{i=1}^{3}\sigma_{ii}^N\left(\frac{3\cos^2\theta_{ii}-1}{2}\right) \quad (2)$$

$$J(\omega_q) = \frac{2}{5}\left(\frac{\tau_c}{1+(\omega_q\tau_c)^2}\right) \quad (3)$$

where γ_C, γ_H, and γ_N denote the magnetogyric ratio of the nuclei ^{1}H, ^{13}C, and ^{15}N, respectively, $r_{C1'H1'}$ is the C1′−H1′ internuclear distance, B_0 is the strength of the magnetic field, $\sigma^N{}_{ii}$ is the *ii*-th component of the diagonalized nitrogen CS-tensor, θ_{ii} is the projection angle between the C1′−H1′ dipole−dipole vector and $\sigma^N{}_{ii}$, and τ_c is the correlation time for isotropic tumbling. $J(\omega_q)$ is the spectral density function at frequency ω_q. In the case of macromolecules at high fields, high frequency terms of the spectral density are small; hence only terms with $\omega_q \approx 0$ are significant. Equation 1 is then reduced to

$$\Gamma_{N1/9,C1'H1'} = \frac{4}{15}\left(\frac{\mu_0\hbar}{4\pi}\right)\frac{\gamma_H\gamma_C}{r_{C1'H1'}{}^3}\gamma_N B_0 \sigma_{N1/9,C1'H1'}\tau_c \quad (4)$$

Experimentally, $\Gamma_{N1/9,C1'H1'}$ is determined from the relaxation of carbon−nitrogen (C−N) double- and zero- quantum coherences.[1,8,9,15] However, it is not possible to separate $\Gamma_{N1/9,C1'H1'}$ from $\Gamma_{C1',N1/9H1'}$ in the experiment (Figure 1a) (for details on experimental setup and theory, see refs 8 and 15). Because of a large N1/9−H1′ interatomic distance of about 2.09 Å and relatively low anisotropy of the C1′ CS-tensor,[12,13] the contribution of $\Gamma_{C1',N1/9H1'}$ is considered to be negligible in practical applications.[8,9]

Computational Methods

The 2′-deoxyadenosine (dAde), 2′-deoxyguanosine (dGua), 2′-deoxycytidine (dCyt), and 2′-deoxythymidine (dThy) were used as model compounds for all calculations of the N1/9 and C1′ CS-tensors. The geometry of all nucleosides was gradient optimized with the B3LYP exchange-correlation functional[16] and the 6-31G(d,p) atomic basis set. In the initial geometry optimization, the χ torsion angle was estimated to be close to either syn or anti local energy minima, and the sugar pucker was adjusted to either C3′-*endo* (pseudorotation angle *P*[17] set to approximately 20°) or C2′-*endo* (*P* about 160°). Subsequently, the constrained geometry optimization for the stepwise change of torsion angle χ was performed for each nucleoside. All geometry parameters were freely optimized except the torsion angle χ. The NMR shielding tensors were calculated using the GIAO approach,[18] with the B3LYP functional and the atomic basis set (9s,5p,1d/5s,1p) [6s,4p,1d/3s,1p] for carbon, nitrogen and oxygen and the (5s,1p) [3s,1p] basis set for hydrogen developed by Kutzelnigg,[19] usually called IgloII. All calculations were done with the Gaussian G03 program.[20]

Taking into account that the glycosidic torsional motion χ is opposed by a nonharmonic potential, the torsional motion is removed from the vibrational problem and considered as a generalized rotation by allowing the molecular valence coordinates to vary with χ. The remaining motions are assumed to be adiabatically separable from the torsional motion, and the appropriate "semirigid-bender" Hamiltonian acquires the following form:

$$\mathrm{H} = \frac{1}{2}\mu_{\chi\chi}(\chi)J_\chi{}^2 + V(\chi) + V_{\mathrm{pseudo}}(\chi) \quad (5)$$

$J(\chi) = -i\hbar(\partial/\partial\chi)$; $V(\chi)$ is the potential energy function obtained from the quantum chemical calculations; $V_{\mathrm{pseudo}}(\chi)$ is the pseudo-potential term arising from the vibrational dependence of $\mu_{\chi\chi}$ (the torsional component of the tensor that is inverse of the 4 × 4 generalized molecular inertia tensor; for details see ref 21).

The state-dependent structural characteristics associated with the measured cross-correlated relaxation rates are obtained by averaging the available CS-tensor (σ) over the pertinent vibrational eigenfunctions. $\sigma^i = \langle\Psi_i(\chi)|\sigma(\chi)|\Psi_i(\chi)\rangle$, where $\psi_i(\chi)$ is the vibrational wave function of a given vibrational state *i*.

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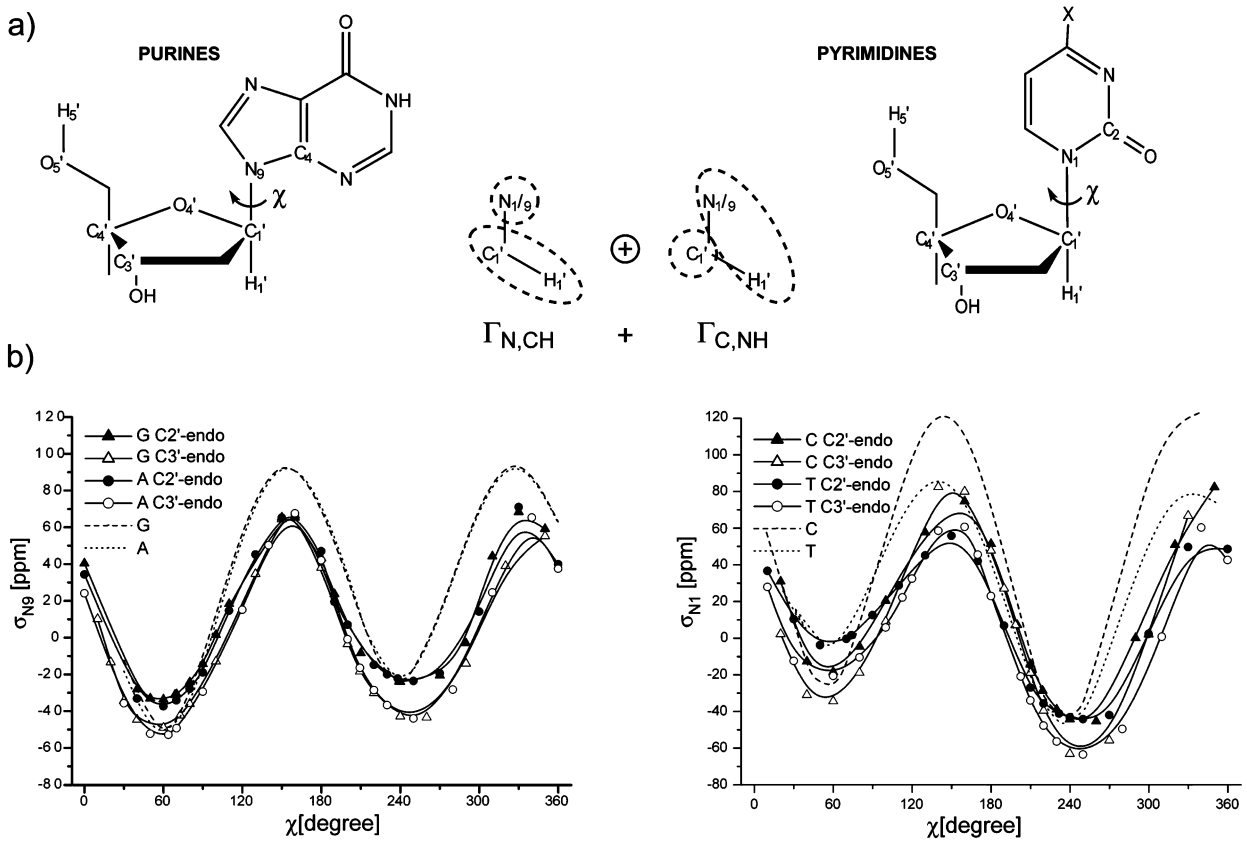

Figure 1. (a) Schematic representations of purine and pyrimidine 2′-deoxynucleosides. The nuclei between which the interactions of the CS-tensor (circles) and dipole−dipole (elipsoid) give rise to experimentally observable cross-correlated relaxation rates[8,9] are highlighted. (b) Solid lines are indicative of the calculated dependence of $\sigma_{N1/9,C1'H1'}$ on glycosidic torsion angle χ for dAde, dGua, dCyt, and dThy. The $\sigma_{N1/9,C1'H1'}(\chi)$ was calculated with eq 2, using sugar and χ specific N1/9 CS-tensor values derived in this study. Dashed and dotted lines indicate modulation in $\sigma_{N1/9,C1'H1'}$ arising from the conformationally independent N1/9 CS-tensor and, therefore, only reflect the dependence of $\sigma_{N1/9,C1'H1'}$ on the angle between the C1′−H1′ bond vector and the principal axes of the N1/9 CS-tensor (eq 2). Dotted and dashed lines were calculated using eq 2 from CS-tensor values taken for C2′-endo sugar pucker and χ equals 250° and 220° for purines and pyrimidines, respectively.

Results and Discussion

Figure 1b shows a sizable dependence of $\sigma_{N1/9,C1'H1'}$ (the term responsible for modulation of $\Gamma_{N1/9,C1'H1'}$, see eq 4) on all of the considered descriptors, i.e., torsion χ, sugar pucker, and DNA base type. $\sigma_{N1/9,C1'H1'}$ is primarily modulated by torsion χ with one minimum for both anti and syn conformations.[22] Both anti and syn minima are steep, 30° rotation around χ changes $\sigma_{N1/9,C1'H1'}$ by about 20 ppm. For pyrimidines, $\sigma_{N1/9,C1'H1'}$ reaches more negative values in the anti region than in the syn region. In the case of purines, the situation is opposite in agreement with the experiment by Duchardt et al.[9] The magnitude mainly depends on the orientation of the C1′−H1′ vector relative to the N1/9 CS-tensor. $\sigma_{N1/9,C1'H1'}$ reaches its minimum for the C1′−H1′ vector lying in the plane of the DNA base. In the anti region, this arrangement coincides with the geometry optimum for C2′-*endo* (∼240°), while the optimal χ for C3′-*endo* is shifted to lower values (∼200°). Significantly, quantum energy minima for different sugar puckers coincide with the minima determined by X-ray crystallography.[23] As is shown in Figure 1b, for purines with C2′-*endo*, $\sigma_{N1/9,C1'H1'}$ is expected to be about 40 ppm lower than $\sigma_{N1/9,C1'H1'}$ for C3′-*endo*. For pyrimidines, the corresponding difference reaches up to 30 ppm. In the absence of extensive local motion, these differences might be exploited to discriminate anti nucleotides in DNA according to its sugar conformation. In the syn region, the geometry optima for C2′-*endo* and C3′-*endo* sugar are essentially the same.

Contrary to generally accepted assumptions,[8,9] our calculations indicate that $\sigma_{N1/9,C1'H1'}$ vs χ for C2′-endo significantly differs from $\sigma_{N1/9,C1'H1'}$ vs χ for C3′-*endo* (Figure 1). In the anti region (for χ ∼240°), up to 20 ppm in $\sigma_{N1/9,C1'H1'}$ is indicated by our calculations. This difference predominantly results from dependency of the magnitude and orientation of the N1/9 CS-tensor on the conformation of the sugar ring (see Supporting Information for details). In addition, $\sigma_{N1/9,C1'H1'}$ notably reflects changes in the N1/9 CS-tensor resulting from reorientation of the glycosidic torsion (see Supporting Information).

The calculated dependencies of the N1/9 and C1′ CS-tensors give rise to a basic question: to what extent is the conformational dependence of these tensors reflected in the experimentally accessible Γ, which is measured as a sum of $\Gamma_{N1/9,C1'H1'}$ plus $\Gamma_{C1',N1/9H1'}$? Figure 2 shows experimental data acquired by Duchardt et al.[9] along with $\Gamma(\chi)$ curves calculated with eq 4 using the N1/9 and C1′ CS-tensor values derived in this study. It has to be emphasized that the experimental data from RNA have been used for the comparison because no experimental data for DNA is available to date. The original Duchardt's

(22) Torsion χ is defined by atoms O4′−C1′−N1−C2 in pyrimidines and by atoms O4′−C1′−N9−C4 in purines. There are two energetically allowed regions of χ, anti and syn. In agreement with comparative studies of crystallographic data, the anti region is defined as 180° < χ < 280°, and syn, as 50° < χ < 80°.

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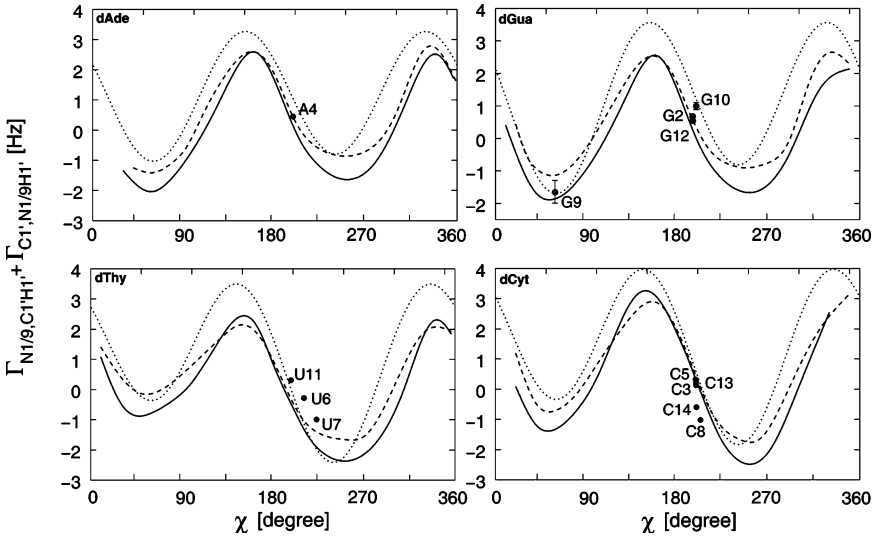

Figure 2. $\Gamma_{N1/9,C1'H1'} + \Gamma_{C1',N1/9H1'}(\chi)$ curves for dAde, dGua, dThy, and dCyt. Dashed and solid lines stand for $\Gamma_{N1/9,C1'H1'} + \Gamma_{C1',N1/9H1'}(\chi)$ curves for C2′-endo and C3′-endo sugar puckers, respectively. The curves were calculated using eq 4, with N1/9 and C1′ CS-tensor values derived in this study with $\tau_c = 2.5$ ns, rC1′−H1′ = 1.09 Å, rN1/9−H1′ = 2.09 Å, and $B_0 = 14.0926$ T. Solid circles represent the experimental cross-correlated relaxation rates acquired from r(GGCACUUCGGUGCC) by Duchardt, plotted against reference χ angles.[9] The dotted line shows the Duchardt's parametrizations taken from ref 9. Duchardt's parametrizations were calculated using experimentally determined N1/9 CS-tensor values for adenosine, guanosine dihydrate, cytidine, and 2′-deoxythymidine.[9] Duchardt's parametrizations assume: (a) interdependency of both magnitude and orientation of N1/9 CS-tensor on local DNA structure and (b) $\Gamma_{C1',N1/9H1'} = 0$.

Table 1. Experimental $\Gamma_{N1/9,C1'H1'} + \Gamma_{C1',N1/9H1'}$ from r(GGCACUUCGGUGCC)[9] Compared to Γ Values Back-Calculated: (i) from Model of Duchardt[9] and (ii) with Eq 4 Using Sugar and χ Specific N1/9 and C1′ CS-Tensors Derived in This Study

res. name	$\Gamma^{expt\ a}$ [Hz]	$\Gamma^{Duchardt}$ [Hz][a]	$\lvert\Gamma^{exp} - \Gamma^{Duchardt}\rvert^{b}$ [Hz]	$\Gamma^{calcd\ c}$ [Hz]	$\lvert\Gamma^{expt} - \Gamma^{calcd}\rvert^{b}$ [Hz]
G2	0.67 ± 0.04	1.48	0.81	0.61	0.06
G9	−1.64 ± 0.35	−1.70	0.06	−1.86	0.22
G10	1.00 ± 0.11	1.21	0.21	0.00	1.00
G12	0.53 ± 0.04	1.48	0.95	0.61	0.08
		r.m.s.d	***0.51***		***0.34***
U6	−0.29	−1.15	0.86	−1.30	1.01
U7	−0.99 ± 0.11	−1.96	0.97	−1.37	0.38
U11	0.29	−0.01	0.3	−0.55	0.84
		r.m.s.d.	***0.71***		***0.74***
C3	0.14 ± 0.01	0.57	0.43	0.30	0.16
C5	0.32 ± 0.07	0.62	0.30	0.35	0.03
C8	−1.03 ± 0.08	0.18	1.21	−0.05	0.98
C13	0.21 ± 0.04	0.57	0.36	0.30	0.09
C14	0.55 ± 0.08	0.57	0.02	0.30	0.25
		r.m.s.d.	***0.46***		***0.30***
A4	0.44 ± 0.01	1.03	**0.59**	0.13	**0.31**

[a] Values were taken from ref 9. [b] Brackets stand for absolute value. [c] Values were calculated as $\Gamma_{N1/9,C1'H1'} + \Gamma_{C1',N1/9H1'}$ with eq 4 for $\tau_c = 2.5$ ns, $B_0 = 14.0926$ T, $r_{C1'-H1'} = 1.09$ Å, $r_{N1/9-H1'} = 2.09$ Å and sugar and χ specific N1/9 and C1′ CS-tensor values given in the Supporting Information. All residues were assumed to have C3′-*endo* sugar pucker except residues U7 and C8. These were assigned with C2′-*endo* sugar pucker according to both NMR (PDB entry 1HLX) and crystallographic (PDB entry 1FY7) data for cUUCGg-tetraloop. r.m.s.d. stands for root-mean-square deviation and is defined as $\sqrt{\Sigma d_i^2/n}$, where d_i is the difference between the i-th pair of experimental and back-predicted data based on theoretical model and n is the number of points.

parametrizations of Γ(χ) were based on ribonucleoside specific N1/9 CS-tensor values. Duchradt's Γ(χ) neglects both the contribution of $\Gamma_{C1',N1/9H1'}$ and the N1/9 CS-tensor dependence on the sugar pucker and χ. Our Γ(χ) curves calculated for 2′-deoxynucleosides, on the other hand, explicitly involve a $\Gamma_{C1',N1/9H1'}$ term and are based on sugar and χ specific N1/9 and C1′ CS-tensors. As can be seen for dGua, dAde, and dCyt, our Γ(χ) curves better agree with experimental data as compared to the model for Γ(χ) proposed by Duchardt (Table 1 and Figure 2). However, no improvement is achieved for uracil residues (Table 1). This may be due to differences between the N1 and C1′ CS tensors of uracil and 2′-deoxythymidine.

The experimental data were acquired from the r(GGCACUUCGGUGCC) hairpin having all residues with C3′-*endo* sugars except residue U7 and C8 having C2′-*endo* sugars.[9] The application of the calculated C2′-*endo* sugar specific Γ(χ) curve for residue C8 is not an improvement compared to Duchardt's model, as the experimental point lies in the region of χ where Duchardt's and C2′-*endo* and C3′-*endo* sugar specific Γ(χ) curves coincide. However, a different situation holds for residue U7 with $\Gamma^{exp} = -0.99$ Hz. While Duchardt's Γ(χ) curve predicts Γ of −1.96 Hz, Γ(χ) curve, accounting for $\Gamma_{C1',N1/9H1'}$ contribution and sugar and χ specific N1/9 and C1′ CS-tensors, gives −1.37 Hz suggesting that sugar specific Γ(χ) curves might be necessary for proper interpretation of Γ^{exp}.

Strictly speaking, the presented rationalization of the conformational dependence of the CS-tensors and its structural implications in terms of geometrically defined property curves (Figure 1) is approximate. A more quantitative analysis must respect dynamic effects, i.e., quantum-mechanical averaging of

the CS-tensors over the torsional motion. To probe these (vibrational) effects, we have solved the appropriate torsional (one-dimensional) Schrödinger equation, $\hat{H}\Psi(\chi) = E\Psi(\chi)$ and evaluated the sought averages $\sigma_{av} = \langle\Psi|\sigma_{N1/9,C1'H1'}(\chi)|\Psi\rangle$ and $\chi_{av} = \langle\Psi|\chi|\Psi\rangle$ for all of the anti conformations of the studied nucleosides. Then, using the calculated $\sigma_{N1/N9,C1'H1'}$ curves, the averages σ_{av} were "inverted" into effective glycosidic bond angles χ_{eff}. For the C3′-*endo* puckers, the effective angles χ_{eff} are found to coincide closely with their quantum mechanical expectation values χ_{av}. In the case of the C2′-*endo* puckers, χ_{eff}'s are found to be uniformly smaller than χ_{av}'s, although only by a very few degrees ($<5°$). Apparently, the vibrational corrections are rather unimportant and can be safely neglected.

To summarize, the conformational dependent variability of N1/9 tensor affects the interpretation of the cross-correlated relaxation rates between the N1/9 chemical shielding tensor and C1′−H1′ dipole−dipole in DNA. For proper interpretation of $\Gamma_{N1/9,C1'H1'}$ in terms of dynamics and conformation of the glycosidic torsion angle, the use of the sugar specific $\Gamma(\chi)$ accounting for $\Gamma_{C1',N1/9H1'}$ contribution seems to be necessary. The utilization of the sugar specific $\Gamma(\chi)$ might be of crucial importance for $\Gamma_{N1/9,C1'H1'}$ interpretation in both canonical and noncanonical DNA structures, such as Z-DNA, DNA quadruplexes, or unusual DNA duplex and hairpin motifs.

Conclusions

The quantum chemical calculations showed that the N1/9 and C1′ chemical shielding tensors depend on the glycosidic bond orientation and sugar pucker in four standard DNA nucleosides. Our calculations suggest that accounting for conformationally dependent variability in these tensors might be important for proper interpretation of cross-correlated relaxation rates between the N1/9 CS-tensor and C1′−H1′ dipole−dipole in DNA.

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Supporting Information Available: Calculated N1/9 and C1′ CS-tensors for dGua, dAde, dCyt, and dThy as a function of χ and sugar pucker. This material is available free of charge via Internet at http://pubs.acs.org.

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