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Experimental and Theoretical Determination of Nucleic Acid Magnetic Susceptibility: Importance for the Study of Dynamics by Field-Induced Residual Dipolar Couplings

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Received May 13, 2004; E-mail: bax@nih.gov

The anisotropic magnetic susceptibility exhibited by nucleic acids in solution causes such molecules to adopt a small degree of alignment in a magnetic field, resulting in residual dipolar couplings (RDC) between nuclear spins.[1,2] RDCs, induced either by magnetic field alignment or by anisotropic media such as dilute liquid crystals,[3] have been successfully exploited to provide precise information on biomolecular structure.[4-6] Critical prerequisites to the interpretation of dynamics of nucleic acids from magnetic field-induced RDCs are accurate reference values of the magnetic susceptibility anisotropies ($\Delta\chi$).[7,8] Previous NMR studies have typically relied upon estimated $\Delta\chi$ values with reported uncertainties of 100% in the cases of thymine and cytosine.[9] Here, we establish much-needed reference values of $\Delta\chi$ for each of the nucleic acid bases on the basis of a combined NMR spectroscopic and DFT investigation and highlight the importance of these values toward the interpretation of RDC data in terms of nucleic acid dynamics and structure.

Given its well-defined solution structure,[10] the Dickerson dodecamer is an ideal candidate for a reliable measurement of total magnetic susceptibility anisotropy. To this end, magnetic field-induced one-bond D_{CH} RDCs were measured from NMR spectra obtained at $B_0^{low} = 11.75$ T and $B_0^{high} = 18.8$ T at 308 K. Splittings ($^1J_{CH} + {}^1D_{CH}$) were extracted from the ^{13}C dimension of natural abundance ^{1}H$-^{13}$C correlation spectra (Supporting Information [SI]).[11,12] A total of 35 base and sugar $^1D_{CH}$ RDCs were measured for well-resolved cross-peaks. The value of $\Delta\chi.S$ for the dodecamer, $-16.3 \pm 0.4 \times 10^{-27}$ J T^{-2}, was extracted by a singular-value decomposition fit of the data to the 1NAJ structure according to the following equation:

$$\{(^1J_{CH} + {}^1D_{CH})^{high} - (^1J_{CH} + {}^1D_{CH})^{low}\}\left[\frac{(B_0^{high})^2}{((B_0^{high})^2 - (B_0^{low})^2)}\right]$$

$$= -\left[\frac{(B_0^{high})^2 \Delta\chi S \gamma_C \gamma_H h}{15kT\mu_0 \pi r_{CH}^3}\right]\left[(3\cos^2\theta - 1) + \frac{3R(\sin^2\theta\cos 2\phi)}{2}\right] \quad (1)$$

where S is the generalized order parameter,[3] γ_X is the magnetogyric ratio of nucleus X, $\Delta\chi$ and R are the anisotropy and rhombicity of the χ tensor, respectively, r_{CH} is the internuclear distance (1.08 Å for base CH and 1.09 Å for sugar CH), and θ and ϕ are the polar coordinates describing the orientation of the CH vector in the principal axis system of the χ tensor.

DFT/GIAO calculations of nucleic acid base χ tensors were carried out using *Gaussian03*[13] with the B3LYP functional and the 6-311++G(3df,3pd) basis set on all atoms. The accuracy of the computations was established by reproducing the experimental gas-phase magnetic susceptibility anisotropies[14] for a test set of six

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Table 1. Calculated Magnetic Susceptibility Tensor Anisotropies and Rhombicities for Nucleic Acid Bases[a]

	$\Delta\chi$/10^{-27} J T^{-2}	R^b		$\Delta\chi$/10^{-27} J T^{-2}	R^b
cytosine	−0.392	0.09	guanine	−0.922	0.15
thymine	−0.411	0.39	purine[c]	−1.552	0.05
uracil	−0.386	0.18	pyrimidine[c]	−0.886	0.05
adenine	−1.304	0.03			

[a] B3LYP/6-311++G(3df,3pd) results. See SI for tensor orientations. [b] $\Delta\chi = \chi_{33} - (1/2)(\chi_{11} + \chi_{22})$ and $R = (\chi_{22} - \chi_{11})/\Delta\chi$ where $|\chi_{33} - \chi_{iso}| \geq |\chi_{11} - \chi_{iso}| \geq |\chi_{22} - \chi_{iso}|$. [c] Geometries are optimized at the B3LYP/6-311++G(3df,3pd) level. Note that in this case purine and pyrimidine refer to the specific molecules with these names and not to a class of molecules.

Table 2. Experimental ($\Delta\chi S$) and Predicted Magnetic Susceptibility Anisotropies, $\Delta\chi$, for Selected Nucleic Acids

	DNA dodecamer[a]	GATA-1/DNA[a]
exptl $\Delta\chi S$	−16.3 ± 0.4	−22.4 ± 3.7
DFT-A[b]	−16.5	−21.9
DFT-B[c]	−15.7	−20.4
Lit. 1[d]	−15.3	−20.4
Lit. 2[e]	−27.2	−33.9
Lit. 3[f]	−23.0	−28.9

[a] Predicted $\Delta\chi$ values are obtained by tensor summation using PDB entries 1NAJ and 2GAT, and are reported in units of 10^{-27} J T^{-2}. [b] $\Delta\chi$ values reported in Table 1 were used, but axial symmetry was assumed. [c] $\Delta\chi$ values and rhombicities reported in Table 1 were used. [d] Base $\Delta\chi$ values from ref 9. [e] Base $\Delta\chi$ values from ref 8. [f] Average base $\Delta\chi$ value used in ref 5.

cyclic molecules with a correlation coefficient of 0.998 (SI), representing a significant improvement over previous calculations.[15] Equally good correlations between experimental and calculated *isotropic* magnetic susceptibilities for a variety of hydrocarbons have been reported by Ruud et al.[15] The deviation from unity of a few percent in the slope of our correlation is consistent with previous studies of isotropic magnetic susceptibilities,[15,16] and is indicative of the limitations in quantitative accuracy of the calculations.

Two different sets of atomic coordinates for nonzwitterionic, keto/amino form nucleic acid bases were employed. The first uses B3LYP/6-311+G* optimized geometries, and the second uses median heavy-atom bond lengths and angles compiled by Clowney et al.[17] The results summarized in Table 1 are based on the first set of geometries. Results based on the second set are within a few percent of the first and are also well reproduced using a restricted Hartree–Fock approach (SI).

The additivity of magnetic susceptibilities is exploited to compare experimental and calculated results. Tensor summation using the values reported in Table 1 and the NMR structure of the dodecamer[10] provide total anisotropies within experimental error of the observed bulk magnetic susceptibility anisotropy (Table 2). This quantitative agreement is despite the fact that the experimental values subsume the effect of internal motions, commonly accounted for by scaling with the order parameter S. The DFT results provide
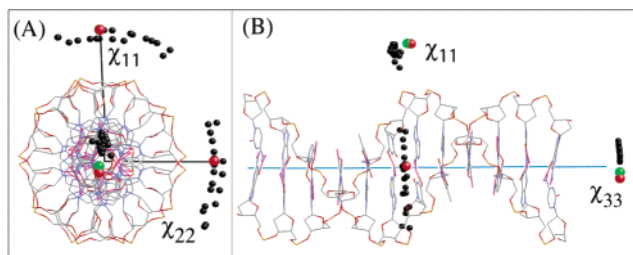
Figure 1. Experimental (black dots) and predicted (green, using axially symmetric χ tensors; red, including rhombicities) DNA dodecamer (d(CGCGAATTCGCG)$_2$) χ tensor orientations. (A) View down the helical axis, with predicted and experimental χ_{33} components within ~4° of this axis. The two predicted χ_{22} components and the two predicted χ_{11} components overlap. (B) View along the predicted χ_{22} component; helical axis is blue. Experimental distribution generated by adding random noise (at the level of the experimental rmsd) to ideal data, and noise-corrupted data then used to extract the magnetic susceptibility tensor. R(expt) = 0.05 ± 0.02; R(pred., axially symmetric χ) = 0.01; R(pred., rhombic χ) = 0.02.

accurate relative values of $\Delta\chi$ for the various bases and, in combination with our experimental data, have predictive value for other systems. The experimental orientation of χ_{11} and χ_{22} are also well reproduced despite the small rhombicity (Figure 1).

The potential contribution of the sugar and phosphate groups to the observed value of $\Delta\chi$ in nucleic acids was also investigated. For a series of sugar−phosphate conformers taken from Murray et al.[18] and from the dodecamer structure, values of $\Delta\chi$ on the order of $\pm0.100 \times 10^{-27}$ J T^{-2} are obtained by DFT (SI). It is clear that the precise magnitude and orientation of χ for the sugar−phosphate moiety are sensitive to sugar conformation and backbone torsion angles and are also expected to be influenced by solvent and/or cation coordination in solution. Given the excellent agreement obtained between experiment and theory in the absence of a term for sugar−phosphate magnetic susceptibility, and the small, imprecise nature of the calculated values, their inclusion in the tensor summations could not be justified, although they may contribute to the experimental $\Delta\chi$ value being slightly larger than the computed one.

The measured value of $\Delta\chi S$ for a protein−DNA complex (GATA-1/DNA),[4] $-22.4 \pm 3.7 \times 10^{-27}$ J T^{-2}, provides a further stringent test of the values in Table 1. Both tensor summation results (assuming axially symmetric χ tensors, or including rhombicities) are well within the experimental error (Table 2). The minor contribution to $\Delta\chi$ from the protein (2%) was included using DFT-calculated magnetic susceptibility anisotropies and rhombicities for peptide bonds as well as aromatic amino acid side chains (SI).

Examination of the discrepancies between Skoglund's empirical values[9] and those reported in Table 1 shows differences to be mainly related to individual bases, whereas the average DNA per-basepair values of $\Delta\chi$ are in reasonable agreement (-1.42 and -1.51×10^{-27} J T^{-2}). This is in contrast to more widely used literature values (Table 2 and SI).

Base susceptibility values are critical in the assessment of biomolecular structure and dynamics from field-induced RDCs. For example, van Buuren et al. derived interhelical angles in a branched nucleic acid by minimizing the difference between experimental RDCs and those predicted from trial conformations and an average $\Delta\chi$ value of -1.03×10^{-27} J T^{-2} per base, or alternatively base-specific values of $\Delta\chi$.[5] Our different base-specific values and lower per-base value can impact upon this process of global structure determination. If the value of $\Delta\chi$ predicted on the basis of base susceptibilities and the known structure exceeds the value derived from experimental RDCs, this has been interpreted as the result of intramolecular motions, which generally will lead to partial averaging and thereby smaller magnitudes of the RDCs.[8] Using

our DFT values to determine $\Delta\chi_{calc}$ for the DNA dodecamer and the GATA-1/DNA complex, we obtain a ratio $\Delta\chi_{expt}S/\Delta\chi_{calc}$ equal to unity (within experimental error), a result anticipated for systems that lack large-amplitude motions. However, using other literature values to determine $\Delta\chi_{calc}$ (Table 2) results in a ratio as low as 0.60, which could be mistakenly interpreted as the result of large-amplitude internal dynamics. Notably, the widely used values of $\Delta\chi$ for pyrimidines[2] actually apply to pyrimidine itself; however, the values for keto-form C, T, and U are substantially lower.[9]

As commercially available magnetic field strengths continue to increase, field-induced residual anisotropic interactions are expected to play an increasingly important role in the NMR study of biomolecules. The use of accurate reference values, derived by computation and validated by experimental methods, is critical in the interpretation of such data.

Supporting Information Available: Figures displaying experimental data for d(CGCGAATTCGCG)$_2$, calculated vs experimental data for a set of test molecules, the χ tensor orientations for nucleic acid bases and for sugar−phosphate conformers. Tables with experimental couplings in d(CGCGAATTCGCG)$_2$, calculations of χ for the bases using alternative methods and geometries, calculations of χ for the peptide plane and some amino acid side chains, a summary of some literature $\Delta\chi$ values, a summary of sugar−phosphate χ calculations. This material is available free of charge via the Internet at http://pubs.acs.org.

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JA047179O