Magnitudes and Orientations of the ^{15}N Chemical Shift Tensor of [1-^{15}N]-2′-Deoxyguanosine Determined on a Polycrystalline Sample by Two-Dimensional Solid-State NMR Spectroscopy

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The magnitudes and orientations of the ^{15}N chemical shift tensor of [1-^{15}N]-2′-deoxyguanosine were determined from a polycrystalline sample using the two-dimensional PISEMA experiment. The magnitudes of the principal values of the ^{15}N chemical shift tensor of the N1 nitrogen of [1-^{15}N]-2′-deoxyguanosine were found to be $\sigma_{11} = 54$ ppm, $\sigma_{22} = 148$ ppm, and $\sigma_{33} = 201$ ppm with respect to (^{15}NH$_4$)$_2$SO$_4$ in aqueous solution. Comparisons of experimental and simulated two-dimensional powder pattern spectra show that σ_{33N} is approximately collinear with the N–H bond. The tensor orientation of σ_{33N} for N1 of [1-^{15}N]-2′-deoxyguanosine is similar to the values obtained for the side chain residues of ^{15}N$_{\epsilon 1}$-tryptophan and ^{15}N$_{\pi}$-histidine even though the magnitudes differ significantly.   © 1999 Academic Press

Key Words: ^{15}N chemical shift tensor; solid-state NMR; DNA; deoxyguanosine.

INTRODUCTION

The N1 nitrogen of deoxyguanosine participates in standard Watson–Crick hydrogen bonding in duplex DNA, as shown in Fig. 1, as well as in Hoogsteen hydrogen bonding in triplex and tetraplex structures (1–9). Nitrogen sites participate as both donors and recipients in the hydrogen bonding of DNA base pairs. Since the nitrogen sites in DNA can be specifically and uniformly labeled with ^{15}N (10, 11), the chemical shift and dipolar interactions can be highly informative about hydrogen bonding and other structural parameters of DNA in both solution (12–14) and solid-state (15–19) NMR experiments.

The magnitudes and orientations of the principal values (σ_{11}, σ_{22}, and σ_{33}) of the chemical shift tensor are essential for the interpretation of chemical shift measurements in all NMR experiments. The traditional way to fully characterize chemical shift tensors is to perform a single crystal rotation study on a molecule whose structure has been determined previously by X-ray diffraction (20, 21). A major drawback to this procedure

is that it can be difficult, or in some cases impossible, to grow and align the large, high-quality crystals needed for the solid-state NMR experiments. As a result, there are a limited number of chemical shift tensors of all types available for analysis (22) and only a handful of ^{15}N chemical shift tensors relevant to the cyclic side chains found in biopolymers, including histidine, tryptophan, uracil, benzamide, five- and six-membered heterocyclic compounds, and substituted pyrazines (15, 23–26).

We have developed an alternative solid-state NMR approach to chemical shift tensor determination that enables the use of polycrystalline instead of single crystal samples (23, 27). Previously, we have utilized this three-dimensional powder pattern technique to characterize the magnitudes and orientations of the ^{15}N chemical shift, ^{1}H chemical shift, and ^{1}H–^{15}N dipolar coupling tensors of a peptide bond, and the nitrogen side chains of the amino acids tryptophan (indole-ϵ1) and histidine (imidazole-π) (23, 27). In this Article, we report the results from a two-dimensional variant of this method that enables the magnitudes and partial orientations of the ^{15}N chemical shift tensor and the ^{1}H–^{15}N dipolar coupling tensors of the N1 nitrogen of deoxyguanosine to be determined. While the two-dimensional experiment limited our ability to define the orientations of the principal elements in the molecular frame of reference, it did enable a very small powder sample of specifically ^{15}N-labeled deoxyguanosine to be utilized.

RESULTS AND DISCUSSION

Experimental and calculated one-dimensional ^{15}N NMR spectra of polycrystalline [1-^{15}N]-2′-deoxyguanosine are compared in Fig. 2. The magnitudes of the principal values of the chemical shift tensor are found to be $\sigma_{11} = 54$ ppm, $\sigma_{22} = 148$ ppm, and $\sigma_{33} = 201$ ppm (± 2 ppm) with respect to (^{15}NH$_4$)$_2$SO$_4$ in aqueous solution. The isotropic chemical shift measured from a cross-polarized magic angle sample spinning spectrum was 133 ppm (± 2 ppm). The calculated average chemical shift tensor value of 135 ppm (± 2 ppm) is in agree-


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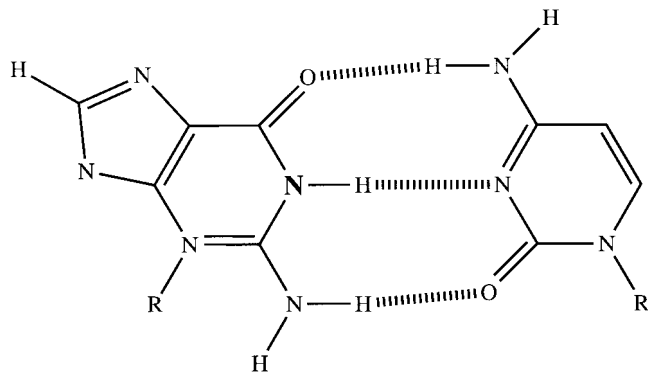
FIG. 1. The GC base pairs in DNA. The hydrogen bonds are illustrated with the elongated dashed bonds. The N1 nitrogen site of deoxyguanosine is shown in boldface.

ment with the experimental value and the isotropic solution value of 130 ppm (adjusted to $(^{15}NH_4)_2SO_4$ in aqueous solution) (*10*).

Experimental and calculated two-dimensional $^1H/^{15}N$ polar-
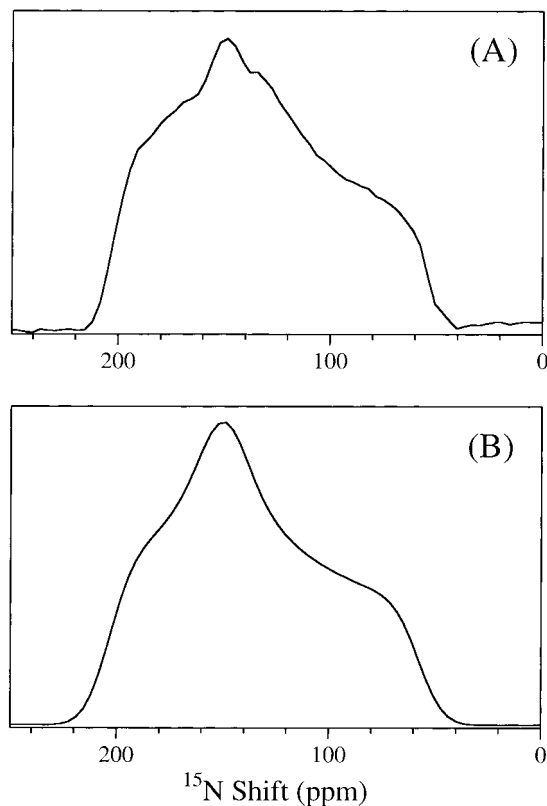

FIG. 2. (A) One-dimensional ^{15}N solid-state NMR powder spectrum of 2 mg of $[1\text{-}^{15}N]\text{-}2'$-deoxyguanosine. The spectrum was obtained via cross-polarization at room temperature with a 1-ms contact time, a 4.0-μs $\pi/2$ pulse, and ^1H decoupling. Four thousand acquisitions were signal averaged with a recycle delay of 7 s. (B) Simulated ^{15}N chemical shift powder pattern spectrum corresponding to $\sigma_{11} = 54$ ppm, $\sigma_{22} = 148$ ppm, and $\sigma_{33} = 201$ ppm.
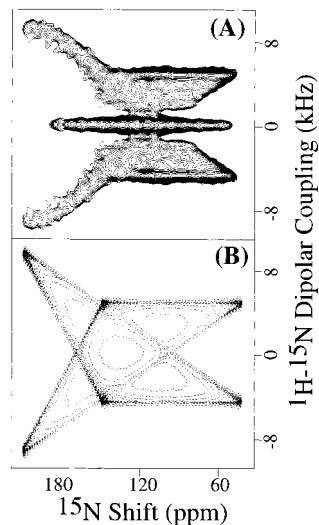
FIG. 3. (A) Two-dimensional ^1H–^{15}N dipolar coupling/^{15}N chemical shift correlation spectrum of 2 mg of a polycrystalline sample of $[1\text{-}^{15}N]\text{-}2'$-deoxyguanosine obtained utilizing the PISEMA pulse sequence. Four hundred sixteen transients were coadded for each of the 48 τ_1 Lee–Goldburg cycles with a dwell time of 32.7 μs. A 7-s recycle delay was used so that the total experiment time was 39 h. (B) Simulated two-dimensional ^1H–^{15}N dipolar coupling/^{15}N chemical shift powder pattern spectrum corresponding to β_N equal to 3° ($\pm 6°$) and α_N equal to 0° (variable).

ization inversion spin exchange at the magic angle (PISEMA) spectra of polycrystalline $[1\text{-}^{15}N]\text{-}2'$-deoxyguanosine are compared in Fig. 3. Two-dimensional PISEMA powder pattern spectra correlate the ^{15}N chemical shift and the ^1H–^{15}N dipolar coupling frequencies for all orientations of the polycrystalline sample. The spectrum was obtained utilizing the two-dimensional PISEMA experiment (*28*), which dramatically increases the spectral resolution in the dipolar coupling frequency dimension compared to standard separated local field experiments. A spectral artifact in the form of intensity centered at 0 kHz in the dipolar fequency dimension can be observed in Fig. 3. This type of artifact generally occurs in PISEMA experiments, when the Lee–Goldberg off-resonance frequency jump is not set exactly, and some magnetization is not spin-locked during the course of the experiment. As in all solid-state NMR experiments it is best to set up the experiment on the sample of interest; however, the small amount of polycrystalline $[1\text{-}^{15}N]$-$2'$-deoxyguanosine available did not allow this. Thus, the zero frequency intensity was ignored in the data analysis.

The experimental two-dimensional PISEMA powder pattern spectrum of $[1\text{-}^{15}N]\text{-}2'$-deoxyguanosine was simulated with multiple parameters in order to determine the orientations of the principal elements of the ^{15}N chemical shift tensor. The ^1H–^{15}N heteronuclear dipolar interaction is assumed to be axially symmetric and collinear with the N–H bond (*20, 21, 30*). The orientations of the principal elements of the ^{15}N chemical shift tensor with respect to the N–H bond vector are defined such that α_N represents the angle between σ_{11N} and
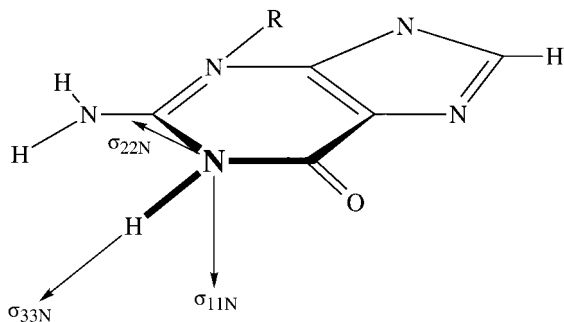
FIG. 4. Orientations of the principal axes of the ^{15}N chemical shift tensor of [1-^{15}N]-2′-deoxyguanosine in the molecular frame. The N1 nitrogen site is shown in boldface.

the projection of the N–H bond vector onto the σ_{11N}–σ_{22N} plane, while β_N represents the angle between σ_{33N} and the N–H bond (23). The magnitudes of the principal values measured from the data in Fig. 1 were utilized in the calculations of the simulated spectra. Two-dimensional spectra corresponding to all possible combinations of α_N and β_N were calculated, and the global minimum difference was found when the angle β_N was close to zero. A good fit to the experimental data was found with β_N equal to 3° ($\pm 6°$) and α_N equal to 0° (variable), and this is the simulated spectrum shown in Fig. 3B.

The simulated spectrum in Fig. 3B and the experimental spectrum in Fig. 3A are similar. This demonstrates that the least shielded ^{15}N chemical shift tensor element (σ_{33N}) is approximately collinear with the N–H bond. The simulated powder patterns are quite sensitive to the angle that β_N makes with respect to the N–H bond vector, and the derived value for β_N is very accurate. Conversely, the qualitative appearance of the simulated spectra does not change dramatically with variation of the α_N angle, and the uncertainty associated with the determination of α_N is substantially larger than that for β_N. The projection of the N–H bond vector onto the σ_{11N}–σ_{22N} plane could be anywhere when β_N has a value near zero; therefore, α_N can have any appreciable value. A series of spectral simulations that illustrates these points has been shown for a complete series of α and β angles for the side chain nitrogens of tryptophan (indole-ϵ1) and histidine (imidazole-π) (23). Also, the spectral simulations indicate that the error associated with making the angle assignments is a very qualitative procedure (23).

The orientations of σ_{11N} and σ_{22N} could not be accurately determined in this study. However, several studies have indicated that σ_{11N} is orthogonal to the plane of the ring, as is the case for aromatic ^{13}C tensor rings (23, 25). By analogy, σ_{22N} would then lie in the plane of the purine ring. The orientations of the principal elements of the ^{15}N chemical shift tensor of deoxyguanosine would then be as shown in the molecular drawing in Fig. 4.

We attempted to obtain the three-dimensional powder pat-

tern that would enable the complete characterization of the magnitudes and orientations of the ^{15}N chemical shift, ^{1}H chemical shift, and ^{1}H–^{15}N dipolar coupling tensors. However, we were unable to obtain data with sufficient signal-to-noise ratios for analysis from the 2 mg of [1-^{15}N]-2′-deoxyguanosine that was available. This is consistent with our previous results, which suggested that 10 mg of a ^{15}N-labeled molecule is necessary to fully characterize the tensors of a stationary polycrystalline sample (23). We would estimate that approximately 1 mg of a polycrystalline sample is needed for a two-dimensional PISEMA analysis of a ^{15}N-labeled molecule. However, less material would be needed for experiments in spectrometers with higher field magnets or at substantially lower temperatures.

EXPERIMENTAL

[1-^{15}N]-2′-deoxyguanosine was synthesized via the transformation of [6-^{15}N]-2′-deoxyguanosine according to the procedure established by Goswami and Jones (10).

All of the solid-state NMR experiments were carried out on a homebuilt NMR spectrometer with a 12.9-T wide-bore Magnex 550/89 magnet at ambient temperatures. The resonance frequencies are 550.9 MHz for ^{1}H and 55.7 MHz for ^{15}N with ^{15}N-labeled ammonium sulfate set to 0 ppm as the chemical shift reference. For the stationary experiments, approximately 2 mg of [1-^{15}N]-2′-deoxyguanosine powder was sealed inside a glass tube and placed inside a double-tuned 2.5-mm 9-turn solenoid coil in a homebuilt probe. The magic angle spinning spectrum was obtained from the same powder sample placed in a double-tuned 5-mm-coil Doty Scientific Inc. probe. Data were processed on a Silicon Graphics O$_2$ computer utilizing the FELIX software package (Biosym) and on a Power Macintosh computer running Igor Pro 3.0 (Wavemetrics).

The two-dimensional solid-state PISEMA pulse sequence was utilized to correlate the ^{15}N chemical shift and ^{1}H–^{15}N dipolar couplings (28). Comparison of results from PISEMA experiments to those obtained with standard separated local field experiments without ^{1}H irradiation during the t_1 interval (29) on a single crystal test sample determined that the experimental scaling factor was equal to 0.82. A spin echo with interpulse delay of 40 μs was used to suppress the effects of probe ringing. A ^{1}H flip-back pulse was added at the end of the sequence to enhance sensitivity because of the relatively long ^{1}H T_1 of the sample. The PISEMA experiments were conducted with RF field strengths corresponding to 62.5 kHz and the $\pi/2$ pulse width was 4.0 μs. The corresponding Lee–Goldburg off-resonance frequency jump was optimized at 44.2 kHz.

Utilizing the conventional notation ($|\sigma_{33}| \geq |\sigma_{22}| \geq |\sigma_{11}|$) for the chemical shift tensor, the principal elements of the ^{15}N tensor were directly measured from the one-dimensional ^{15}N chemical shift spectrum. These experimental values were op-

timized by simulating the powder spectrum with the computer simulation program SOLIDS on a 200-MHz (Pentium chip) PC clone (*32*). The two-dimensional powder pattern spectrum corresponding to the ^{15}N chemical shift and ^1H–^{15}N dipolar couplings was simulated using a Monte Carlo method to generate the entire range of possible orientations that the N–H bonds could take with respect to the direction of the magnetic field. There were 10^6 calculations utilized for each spectral simulation. Additional iterations showed no significant change in the shape or intensity of the simulated spectrum. ^{15}N linewidths of 4 ppm were used in the simulations of the two-dimensional PISEMA spectrum.

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