solution NMR spectroscopy in eliminating broadening from ^{14}N relaxation effects.[34]

Acknowledgment. We thank George Furst for helpful discussions. This research is being supported by grants from the National Institute of Health (GM 24266) and the American Cancer Society (NP-225). T.A.C. is supported by a Cell and Molecular Biology Training Grant. S.J.O. is a Fellow of the A. P. Sloan Foundation (1980-1982).

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N-H Bond Lengths in DNA

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Received November 5, 1981

Because of its central role in biology, the structure of DNA is of substantial interest and has been the subject of numerous investigations. X-ray diffraction studies of oriented polymer fibers[1,2] and crystals of oligonucleotides[3] have described the conformations of DNA in considerable detail based on the positions of the C, N, O, and P atoms. In spite of the importance of interstrand hydrogen bonds to the structure and biological roles of DNA, little is known about the locations of the hydrogens between the base pairs because of the limitations of X-ray diffraction. Both X-ray[4] and neutron[5] diffraction have been used to measure N-H bond lengths of hydrogen-bond donors; however, the results are from crystals of methylated bases participating in Hoogsteen rather than Watson-Crick base pairs.

NMR spectroscopy is well suited for structure determinations, especially in locating the positions of protons, through the spatial dependence of internuclear dipole-dipole couplings.[6] The large number of dipolar interactions in complex chemical systems result in severe spectral overlap among sites and couplings. Separated local field spectroscopy uses chemical shift positions to distinguish among individual sites and selective averaging techniques to measure the heteronuclear dipolar couplings at those sites.[7] The dipolar interactions between carbons and protons have been characterized with this approach in single crystals,[8] oriented fibers,[9] and powder samples.[10,11]

Uniformly ^{15}N labeled DNA gives solid-state NMR spectra with resolved resonances for nearly all nitrogen sites;[12] in particular, both the hydrogen-bond donor and acceptor nitrogens of the adenine-thymine (AT) and guanine-cytosine (GC) base pairs can be distinguished. By determination of the size of the ^{15}N-^{1}H dipolar couplings of these sites and hence the bond lengths, the hydrogen bonds can be fully characterized as long as they are assumed to be linear. Because magic-angle sample spinning is used to average the chemical shift to its isotropic value in the unoriented polymer samples, the ^{15}N-^{1}H dipolar spectra are in the form of Pake powder patterns modulated by the spinning frequency and are observed as dipolar sidebands.[11]

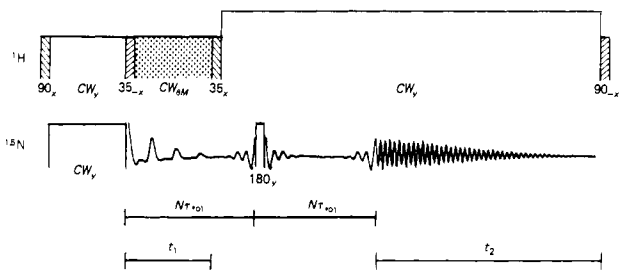

Figure 1. Schematic outline of the pulse sequence used for magic-angle sample spinning separated local field spectroscopy. ^{1}H represents the procedure applied at the proton resonance frequency: P_x are pulses of rotation angle P with relative phases $x = 0°$ or $-x = 180°$; CW_y is continuous irradiation on resonance of phase $y = 90°$ relative to x; $CW_{\theta M}$ is continuous irradiation off-resonance for magic-angle decoupling as described by Lee and Goldburg.[14] ^{15}N represents the procedures applied at the nitrogen resonance frequency: the continuous on-resonance mixing irradiation (CW) and the 180° pulse have the same phase. The free-induction decays are actual experimental data from a molecule with a single nitrogen drawn to scale, except for the length of interval t_2. τ_{rot} is the time for one sample rotation and N is an integer. t_1 and t_2 are the two sampling intervals.

The experimental procedure for separate local field spectroscopy with magic-angle sample spinning is outlined in Figure 1. Initial ^{1}H magnetization is developed in the static magnetic field and then spin locked on resonance with a 90° pulse followed by continuous irradiation shifted in phase by 90°. The ^{15}N magnetization is prepared by simultaneously applying a mixing pulse under Hartmann-Hahn matching conditions.[13] The initial ^{15}N magnetization is allowed to precess for the time interval t_1, which is systematically incremented in the course of the experiments. During the t_1 interval, spin diffusion among the protons is suppressed by decoupling the ^{1}H-^{1}H dipolar interactions with a radiofrequency field applied off-resonance, such that the effective field in the rotating frame points along a direction inclined at the magic angle with respect to the static magnetic field.[14] This magic-angle decoupling scales the ^{15}N-^{1}H dipolar coupling by $3^{-1/2}$. The dipolar precession is monitored during time interval t_2, when fully ^{1}H decoupled ^{15}N isotropic chemical shift free-induction decays are recorded. Additional ^{1}H pulses are inserted in the sequence to preserve coherence of the ^{1}H magnetization in the rotating frame and to flip back any ^{1}H magnetization not transferred to the ^{15}N spins or lost to $T_{1\rho}$ processes to the laboratory frame for optimal sensitivity of the experiment. The ^{15}N 180° pulse refocuses the chemical shift precession, so that pure ^{15}N-^{1}H dipolar spectra are obtained. The entire procedure is synchronized with the sample rotation, such that the ^{15}N 180° pulse and the start of interval t_2 are integral multiples of the sample rotation period.

The spectra in Figure 2 are obtained by Fourier transformation of free-induction decays associated with time intervals t_1 (dipolar couplings in kHz) and t_2 (chemical shift in ppm). The isotropic chemical shift spectra are of hydrated B form DNA[15] (Figure 2A) and low-humidity DNA[16] (Figure 2B). The contour plots in Figure 2C represent the intensities of the dipolar sidebands aligned with the chemical shift positions of Figure 2B. The qualitative correlation of chemical shift with the magnitude of the dipolar

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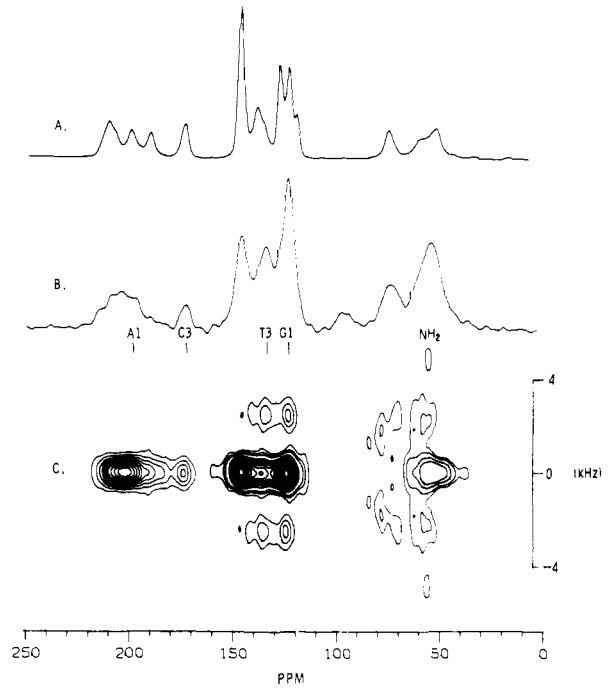

Figure 2. ^{15}N NMR spectra of DNA. (A) Isotropic chemical shift spectrum of B form DNA. (B) Isotropic chemical shift spectrum of low-humidity DNA. Both A and B were obtained at a resonance frequency of 15.24 MHz on a home-built double-resonance spectrometer by cross polarization with 4-ms mix times for 4000 transients with ^1H decoupling (1.5 mT) during data acquisition (50 ms) and magic-angle sample spinning (2.2 kHz). The chemical shifts of the nitrogens discussed in the text are labeled below Figure 2B. (C) Contour plot of dipolar spectra associated with B, obtained by using the pulse sequence in the Figure 1. t_1 was incremented in 30-μs intervals for 64 points, each of which had 2000 transients accumulated during t_2; $N = 4$ and $\tau_{rot} = 0.59$ ms.

sidebands enables the nitrogen sites to be divided into those with substantial intensity in the sidebands having directly bonded hydrogens ($-NH_2$ 40–90 ppm; −N−H 122, 142 ppm) and those with only central zero-frequency intensity having no nearby hydrogens. The quantitative analysis of the sideband intensities[18] by comparison of experimental and calculated dipolar spectra for individual chemical shifts gives the magnitude of the dipolar coupling and hence the N−H bond length. Figure 3A contains the experimental dipolar spectra for the G1 and C3 nitrogens, which are hydrogen bonded in Watson–Crick base pairs. The dipolar spectrum associated with the chemical shift of 124.4 ppm has centerband intensity from the partial overlap of C1 and T1 nitrogens as well as the G1 site; however, since both C1 and T1 are nonprotonated nitrogens the only source of sideband intensity is the G1 nitrogen. The dipolar spectrum associated with the chemical shift of 174.1 ppm is the resolved C3 nitrogen resonance. The calculated dipolar spectra are for three possible locations of the hydrogen between the established N–N distance of 2.92 Å.[17]

Clearly the G1 nitrogen has a directly bonded hydrogen, and the C3 nitrogen does not have a hydrogen nearby. By comparison of the experimental first- and second-order sideband intensities to those calculated for rigid $^{15}N-^1H$ pairs, a value of 1.1_3 Å is obtained for the N−H bond length of G1 of B form DNA. The experiment is capable of very high precision because of the r^{-3} dependence of dipolar coupling on internuclear distance; however, both the accuracy and precision in the determination of bond length are limited by the reproducibility of the experimental scaling factor for the heteronuclear dipolar interaction that is a consequence of the decoupling procedure applied during time interval t_1 to suppress spin diffusion.[14] Within experimental error, the G1 and T3 nitrogens of both hydrated and low-humidity forms of DNA have the same N−H bond length of 1.1_3 Å. This appears

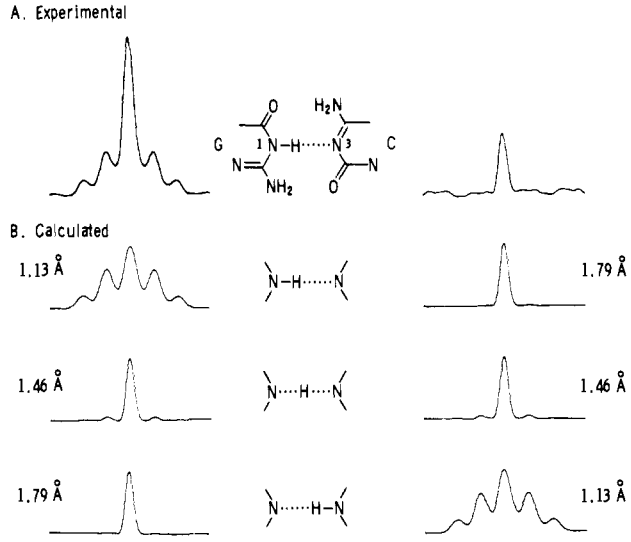

Figure 3. $^{15}N-^1H$ dipolar spectra in the form of spinning sidebands for individual chemical shift positions. The plots are ±6 kHz. (A) Experimental results for B-DNA as described for Figure 2. The left spectrum is from the G1 position (124.4 ppm) and the right spectrum from the C3 position (174.1 ppm). (B) Calculated spectra for the N−H bond lengths listed. The determination of the N−H distance is based only on the sideband intensities of the G1 spectrum in A because the central zero frequency component has intensity from several overlapping nonprotonated nitrogen resonances.

to be in good agreement with the values determined for crystals of model compounds by X-ray[4] (1.09 Å and neutron[5] (1.04 Å) diffraction, especially since small-amplitude vibrational and librational motions are manifested differently in the results of the various determinations. ^1H NMR relaxation rates of DNA fragments in solution are also consistent with bond lengths near 1.0 Å.[19]

The observed N−H bond length means that the hydrogen is localized on the hydrogen-bond-donor nitrogen sites (G1, T3) and not significantly shared with the acceptor nitrogen sites (C3, A1). This is confirmed by the lack of sideband intensity in the C3 and A1 nitrogen dipolar spectra. If these bases have large-amplitude motions on the 10^4-Hz time scale, then the measured N−H bond length would be too long as a consequence of the reduced heteronuclear dipolar coupling. Such motions are absent in both B form and low-humidity DNA, as seen in the lack of motional averaging of the $^{15}N-^1H$ dipolar and $C-^2H$ quadrupole interactions[20] of the bases.

Hydration of DNA does not change the dynamics or the N−H bond lengths of the bases, yet there is a significant effect on the line widths of the ^{15}N base resonances as shown by the comparison of Figure 2 parts A and B. These line widths may reflect a distribution of chemical shifts that is narrower in hydrated DNA. Alternatively, the motions induced by hydration in the backbone of the polymer[20] may be effective in reducing the $^1H-^1H$ dipolar couplings, making the heteronuclear decoupling more efficient in line narrowing. Similar effects of hydration have been observed in ^{13}C and ^{15}N NMR spectra of a variety of solid biopolymers.[21,22]

Acknowledgment. We thank T. A. Cross and M. H. Frey for help with the experiments and calculations and H. Berman for helpful discussions. This research is being supported by grants from the National Institutes of Health (GM-24266) and the American Cancer Society (NP-225). S.J.O. is a Fellow of the A. P. Sloan Foundation (1980–1982).

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