An Alternative Triple-Resonance Method for the Through-Bond Correlation of Intranucleotide H1′ and H8 NMR Signals of Purine Nucleotides. Application to a DNA Dodecamer with Fully $^{13}C/^{15}N$-Labeled Deoxyadenosine Residues

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The ^1H NMR spectra of nucleic acid oligomers show rather limited chemical shift dispersions as compared with those of proteins, and the concomitant signal overlap makes spectral analysis difficult. Recent progress in preparing isotopically labeled RNA oligomers by enzymatic synthesis from ^{15}N- and/or ^{13}C-labeled NTPs provides a new method for NMR analysis of RNA.[1,2] Several groups have applied heteronuclear multidimensional NMR methods for labeled RNAs to establish the sequential signal assignments.[3-6] In particular, ^1H, ^{13}C, and ^{15}N triple-resonance correlation experiments published recently are useful for identifying the intranucleotide H1′–H8/H6 connectivities,[7-9] which had been established only by using through-space connectivities. In this communication, we describe a related but simpler NMR experiment for identifying intranucleotide H1′–H8 correlation in a DNA dodecamer with uniformly $^{13}C/^{15}N$-labeled 2′-deoxyadenosines, using a "shortcut" coherence transfer step from N9 to H8 through the relatively large two-bond J coupling between these two nuclei.

Figure 1 depicts our pulse sequence used to correlate the H1′ and H8 signals in a two-dimensional spectrum. The overall appearance of the sequence resembles the H(CA)NNH described by Kay *et al.* and its derivative sequences.[10-12] In the case of purine nucleosides, the H1′ magnetization is transferred in three steps along the pathways shown in Figure 2. The point distinguishing the present sequence from the previously published schemes[7,9] is that it bypasses the magnetization transfer step to the purine C8, and thus the complex heteronuclear J-coupling networks among the carbon and nitrogen nuclei in the purine bases do not need to be considered.

The pulse scheme of Figure 1 works as follows.[13] A concatenated INEPT sequence transfers the H1′ magnetization to its attached C1′ during the evolution times t_1 and $2\tau_a$.[10,14] The following delay, $\tau_b = 1/2J_{C1',H1'}$, is applied to refocus the C1′ coherence, which is antiphase with respect to the H1′ at the start of the delay, τ_b. After the C1′ coherence is refocused, a WALTZ16[15] broad-band decoupling is applied in a synchronous manner to maintain the in-phase component of the C1′ magnetization, which has a significantly longer relaxation time than the antiphase magnetization. During the interval, $2\tau_c$, the in-phase $C1'_x$ magnetization evolves into the antiphase coherence $2N9_z$-$C1'_y \sin(2\pi J_{N9,C1'}\tau_c)\cos(2\pi J_{C1',C2'}\tau_c)$, where $J_{C1',C2'}$ is about 35 Hz and $J_{N9,C1'}$ is around 11 Hz. To compromise the coherence transfer efficiency from C1′ to N9 and to minimize the attenuation

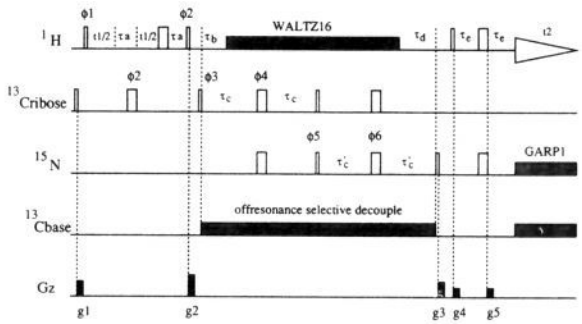

Figure 1. 2D ^1H, ^{13}C, and ^{15}N triple-resonance pulse sequence, H1′C1′N9H8. The narrow and broad bars represent the nonselective 90° and 180° pulses, respectively. Pulses for which the phases are not indicated are applied along the x-axis. The delay intervals are set to τ_a = 1.3 ms, τ_b = 2.6 ms, τ_c = 12.8 ms, τ_c' = 16.2 ms, τ_d = 16.0 ms and τ_e = 8.0 ms. All carbon pulses are generated using a single synthesizer without frequency switching. The coherence delay times, τ_d and τ_e, were experimentally optimized to compensate for the sensitiviey loss by the relaxation effect. A delay time as short as 16 ms was found to be a good value, although it was substantially shorter than the theoretically optimal τ_d of 42 ms. The frequency offset for carbon pulses is at the center of the C1′ carbons, 84.0 ppm. The radio frequency field strength for all carbon pulses is 19.2 kHz. For offresonance selective decoupling, a G3-MLEV16 expansion decoupling pulse is used,[20] where each G3-selective inversion pulse[17] is phase modulated to shift its inversion center to +7.8 kHz,[18,19] which is around the center of the base carbons, 146.0 ppm, excluding the C5 carbon. This G3-MLEV16 selective decoupling is achieved at a field of 2.9 kHz, and under these conditions the selective decoupling has a bandwidth of ±1.5 kHz and has little perturbation on the deoxyribose ring carbons. The ^1H pulses are at a field strength of 29.5 kHz, with an offset on the water resonance. For ^1H decoupling, WALTZ16[15] is used with a 2.7-kHz field strength. All nitrogen pulses are applied at a field strength of 6.6 kHz, with an offset at the midpoint between the N9 and N7 nitrogen resonances, at 180.0 ppm. During acquisition, GARP1[22] decoupling is applied from the nitrogen channel at a field strength of 0.86 kHz and, simultaneously, the same ^{13}C selective decoupling as described above is applied from the carbon channel. The durations and strengths of the gradients are $g1$ = (1.0 ms, 8.0 G/cm), $g2$ = (4.0 ms, 28.2 G/cm), $g3$ = (3.0 ms, 18.3 G/cm), and $g4 = g5$ = (1.0 ms, 5.0 G/cm). A delay of at least 150 μs is inserted between the gradient pulse and the subsequent application of a radio frequency pulse to avoid the eddy current effects. All gradients are applied along the z-axis and are rectangular. The phase cycle is $\phi1 = x$, $\phi2 = y, -y$, $\phi3 = x$, $\phi4 = 2(x), 2(y), 2(-x), 2(-y)$, $\phi5 = 8(x), 8(-x)$, $\phi6 = x$; receiver = $x, 2(-x), 2(x), 2(-x), x, -x, 2(x), 2(-x), 2(x), -x$. The quadrature detection in t_1 is accomplished by States-TPPI[29] of $\phi1$.

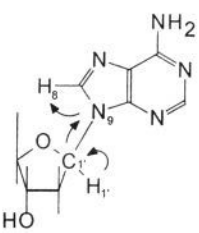

Figure 2. Coherence transfer pathway of the H1′C1′N9H8 experiment for the purine deoxyribonucleotide moiety.

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caused by both passive C1′–C2′ coupling and the relaxation effect, τ_c was set to 12.8 ms. At the beginning of the $2\tau_c$ interval, base carbons are selectively decoupled so that ^{15}N9 magnetization is not affected by the complex ^{15}N–^{13}C coupling network in the base moiety. For this purpose, in the present experiment we applied a selective decoupling pulse[16] in which a Gaussian pulse cascade, G3,[17] with a phase gradient to shift the decoupling center to +7.8 kHz,[18,19] is utilized in the MLEV16 expansion.[20] In the following $2\tau'_c$, the antiphase N9 polarization is allowed to refocus with respect to the C1′ spin, and during the last overlapped delay, τ_d, the N9 magnetization simultaneously becomes antiphase with respect to H8 through the relatively large two-bond J-coupling between N9 and H8, which is ~12 Hz.[21] The N9 coherence is then transferred to H8 in a reverse INEPT step.

During the detection period, the C8, N7, and N8 nuclei interacting with H8 through the large spin coupling are simultaneously decoupled using GARP1[22] for ^{15}N and the off-resonance selective decoupling for ^{13}C. A few field gradient pulses were also used in this experiment. The first gradient pulse is applied after the first ^{13}C $\pi/2$ pulse to ensure that signals from ^{13}C do not exist during the t_1 evolution and $2\tau_a$.[23] To purge unwanted coherence, two gradient pulses, $g2$ and $g3$, were applied when the magnetization of interest was converted into zz-orders.[23] The gradient pulse pair $g4$ and $g5$ is used to generate a perfect echo.[24,25] This pulse sequence produces a 2D NMR spectrum that correlates the H1′ resonances in the F_1 dimension to the H8 resonances in the F_2 dimension. We refer to this pulse sequence as *H1′C1′N9H8*.

A 2D H1′C1′N9H8 spectrum of d(CGCGAATTCGCC)$_2$ with two fully ^{13}C- and ^{15}N-labeled deoxyadenosine residues[26] is shown in Figure 3. Two H1′-H8 correlation signals, which correspond to the A5 and A6 residues, are clearly observed in the spectrum. The ^{1}H NMR assignment of this dodecamer was already established by Hare *et al.*,[30] and we confirmed their assignment using labeled dodecamers. It should be noticed that the spectrum in Figure 3 was measured with 2 h using 160 μL of 1.4 mM solution of the labeled dodecamer in a Shigemi microcell.[31] The high sensitivity obtained by the present method may be partly due to the smaller number of pulses in the sequence as compared to the existing methods, in which selective and/or semiselective pulses are required to suppress the attenuation of the magnetization due to spin coupling between N9 and C4. In our case, however, during the magnetization transfer step from N9 to H8, the scalar coupling effects caused by $J_{N9,C4}$ and $J_{N9,C8}$ can be completely suppressed by a selective decoupling of the base carbons. Another factor that may contribute to the increased sensitivity of the present method is that, the lossy stages with magnetization at the protonated ^{13}C, C8 for purines and C6 for pyrimidines, are replaced by those at the nonprotonated ^{15}N, N9 for purines and N1 for pyrimidines. The method, however, may not work as efficiently for the pyrimidine nucleotides as for purines. Taking the ^2J values for pyrimidines, ~7 Hz, into consideration, the present sequence is less sensitive for pyrimidines than for purines, and therefore the previous sequences utilizing the larger $^1J_{N6,C6}$, 13 Hz, may be a better choice for pyrimidine residues. The H1′C1′N9H8 experiment can be applied to deoxyguanosine residues and to purine nucleotides in RNA oligomers as well, since all of the relevant coupling constants in the magnetization transfer steps are almost identical for ribo- and deoxyriboadenosine and guanosine.

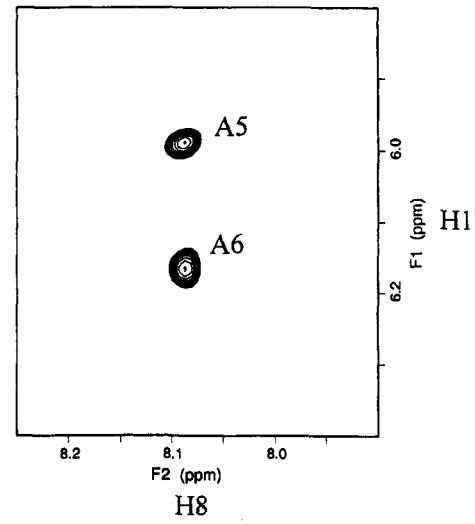

Figure 3. 2D H1′C1′N9H8 spectrum of d(CGCGAATTCGCG)$_2$ at 35 °C. Sample preparation is described in ref 26. The data set was recorded as a 512 (t_2) × 96 (t_1) complex matrix. The spectral widths for F_1 and F_2 were 3000.0 and 6024.0 Hz, respectively. For the present data acquisition, we applied 32 scans per t_1 increment, with a relaxation delay of 0.9 s, giving rise to a total measuring time of about 2 h. After data processing with zero-filling in both time domains, the size of the resultant data matrix was 2048 (F_2) × 1024 (F_1) real matrix. The assignments of the observed adenosine H1′–H8 cross peaks were established using the dodecamer with a labeled adenosine at a single site (A6), and they were identical to those reported by Hare *et al.*[30] This experiment was performed on a Varian UNITY$^+$ 500 spectrometer, using 499.84 MHz for proton resonance, equipped with a pulsed-field gradient accessory and a triple resonance probe with an actively shielded z gradient.

After we submitted this communcation, two other methods to correlate H1′ to H8 were published, both of which utilize a direct magnetization transfer step between H8 and N9.[32,33] In these methods, however, the H1′ and H8 correlation is not achieved via the efficient coherence relay method described in the present paper, and, therefore, the correlation is either indirect[32] or too complex and thus less sensitive.[33]

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