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Through-Bond Correlation of Sugar and Base Protons in Unlabeled Nucleic Acids

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This work presents two methods for through-bond correlation between sugar and base protons in view of model-independent assignment in unlabeled or slightly enriched nucleic acids. Each method uses a combination of multiple-bond and one-bond heteronuclear *J*-couplings to the aromatic carbon C6 for pyrimidines ($^3J_{H1',C6}$ and $^1J_{H6,C6}$) or C8 for purines ($^3J_{H1',C8}$ and $^1J_{H8,C8}$). The techniques are demonstrated in the duplex [d(CGCGAATTCGCG)]$_2$ and the dimeric G-quadruplex [d(GGGTTCAGG)]$_2$ at natural abundance.

Key Words: **heteronuclear NMR; spectral assignment; through-bond correlation; unlabeled nucleic acids; low isotopic abundance.**

INTRODUCTION

Spectral assignment is a decisive step in NMR studies of nucleic acids. For model-independent assignment, through-bond correlation techniques are usually required. Together with correlations in the sugar and in the base, through-bond correlations between sugar and base protons identify resonances belonging to the same nucleotide (*1, 2*). This completes the specific assignment procedures, which start from sequential connection of ^{31}P and sugar protons (*3*), or from base resonance identification by isotopic or chemical substitutions (*4 , 5*). Many techniques for H1′–H6/8 correlation have been proposed for ^{13}C/^{15}N-labeled nucleic acids (*6–17*). However, isotopic labeling is still expensive and presents undesirable couplings between heteronuclei. This provides a motivation for developing hetero-correlation methods applicable to unlabeled molecules and involving therefore only one carbon or nitrogen (*18–23*). Such methods also apply to weakly random-enriched molecules, which are now popular for avoiding the complication of dipolar and scalar interactions between heteronuclei. We present here two sensitive methods for H1′–H6/8 correlation using a combination of the multiple-bond and one-bond couplings, $^3J_{H1',C6/8}$ (∼3–6 Hz) and $^1J_{H6/8,C6/8}$ (∼180–220 Hz) (Fig. 1A), where the value of the former has been reported for both *anti* and *syn* glycosidic conformations (*18, 19*). The methods are demonstrated for the (24-nucleotide) Dickerson duplex [d(CGCGAATTCGCG)]$_2$ and the (18-nucleotide) dimeric G-quadruplex [d(GGGTTCAGG)]$_2$ at natural abundance.

RESULTS AND DISCUSSION

(a) In the first method, the H1′ proton and the H6/8 proton are correlated to carbon C6/8 by a multiple-bond and a one-bond experiment, respectively. The one-bond H6/8-C6/8 correlation (Fig. 2A) is easily obtained with the standard HSQC sequence (*24*). The multiple-bond H1′–C6/8 correlation is more difficult to obtain due to relaxation and to homonuclear *J*-couplings of H1′ with other sugar protons during the long transfer delays. In order to reduce the effect of relaxation, the transfer delays are optimized (*22, 23*). As for the effect of homonuclear *J*-couplings of H1′, this is eliminated by the use of a 180° H1′-selective pulse (*25*) in the center of each INEPT period (*26*), as shown in the selective HSQC scheme (sHSQC) (Fig. 1B).

In the 24-h sHSQC spectrum shown in Fig. 2B, all H1′–C6/8 cross peaks are observed. Combination with the one-bond HSQC spectrum (Fig. 2A) provides correlations between H1′ and H6/8 via the ^{13}C frequency. These correlations confirm the assignments obtained previously (*27*) by NOESY spectroscopy. Note that the intra-base H5–C6 cross peaks (labeled with an asterisk, Fig. 2B) are observed for cytidines since H1′-selective pulses are effective also at the H5 proton frequency.

The multiple-bond H1′-C6/8 correlation can also be obtained in a multiple-quantum experiment. The multiple-bond selective HMQC (sHMQC) (Fig. 1C) is derived from the HMQC and the HMBC sequences (*28*) by the addition of 180° H2′/2″-selective pulses (Fig. 1C), which eliminate the main homonuclear *J* modulation of H1′ during the dephasing and rephasing delays. It is worth mentioning that only two hard pulses on H1′ are used. A filter ^{13}C pulse, applied at time δ to suppress one-bond correlations, is optional. The sHMQC spectrum (Fig. 2C) is somewhat more sensitive than the sHSQC one (Fig. 2B, plotted at the same level) probably because the former uses fewer pulses. The difference in sensitivity and in linewidths

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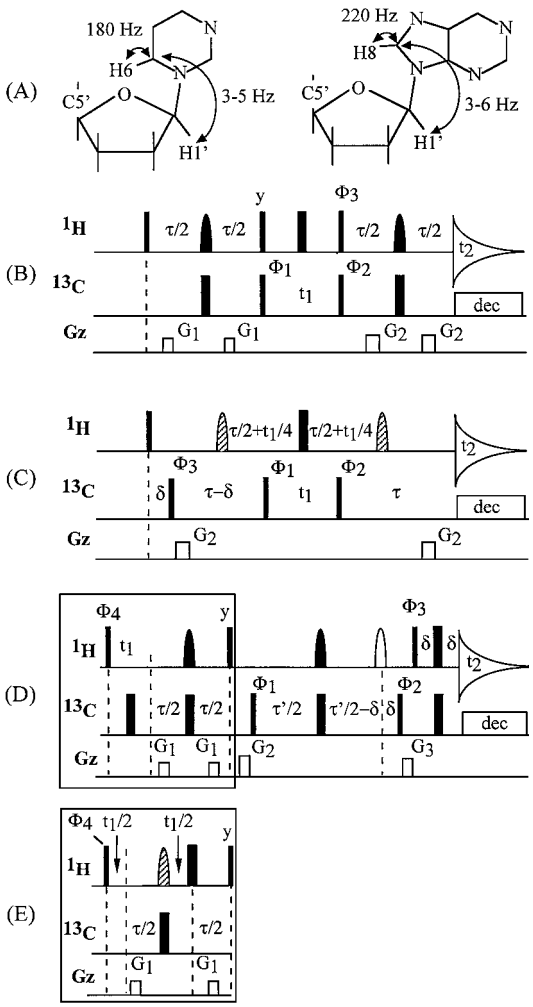

FIG. 1. (A) Schema of pyrimidine (left) and purine (right) nucleotides. Arrows indicate the coherence transfer pathways used in the experiments. (B and C) sHSQC and sHMQC pulse sequences for multiple-bond ^{1}H–^{13}C correlation. (D and E) HCH pulse sequences for the ^{1}H–^{1}H correlation through carbon. First and second lines, RF events; third line, *z*-axis pulsed-field gradient events. RF filled rectangles represent 180° (wide) and 90° (narrow) hard pulses; ovals represent 180° selective pulses on H1′ (black), H6/8 (white), and H2′/2″ (hatched) protons. The selective pulses were generated by a frequency-shifted DANTE sequence (*35*). Unless indicated otherwise, pulses are applied along the *x*-axis. Phase cycling: (B) $\Phi_1 = x, -x$; $\Phi_2 = 2(x), 2(-x)$; $\Phi_3 = 4(x), 4(-x)$; receiver $= x, -x, -x, x, -x, x, x, -x$. (C) $\Phi_1 = x, -x$; $\Phi_2 = 2(x), 2(-x)$; $\Phi_3 = 4(x), 4(-x)$; receiver $= x, -x, -x, x$. (D, E) $\Phi_1 = x, -x$; $\Phi_2 = 2(x), 2(-x)$; $\Phi_3 = 4(x), 4(-x)$; $\Phi_4 = x$; receiver $= x, -x, -x, x, -x, x, x, -x$. In addition, Φ_1 (for B and C) and Φ_4 (for D and E) are phase-cycled in hypercomplex mode (*36*). Gradients: $G_1 = (0.5$ ms, 9 G/cm); $G_2 = (0.5$ ms, 20 G/cm); $G_3 = (0.5$ ms, 15 G/cm). Delays: $\tau = 66$ ms; $\tau' = 72$ ms; $\delta = 2.5$ ms for (C) and $\delta = 1.25$ ms for (D).

for the indirect dimension depends also on relaxation rates of multiple-quantum and single-quantum coherences (*29*, *30*). Another difference between the two spectra is that the sHMQC spectrum (Fig. 2C) is simpler, because the intra-base H5–C6 cross peaks are negative due to unsuppressed $J_{H5,H6}$ modulation. The modulation, which can cause signal loss in case of peak overlap, may be eliminated by application of H6-selective pulses (not shown).

The sHMQC method has been tested at low sample concentration for the dimeric G-quadruplex [d(GGGTTCAGG)]$_2$ which contains G-tetrads sandwiched between G.(C-A) triads (*31*). Most of the H1′–C6/8 correlations are observed (Fig. 3) and confirm the previous assignments (*31*). These correlations are weak or absent in case of *syn* conformations (guanosines 1 and 8), for which the H1′ and H6/8 protons can be correlated via C2/4 (Figs. 3C, 3D). The H1′–C2/4 correlations provide evidence of the presence of *syn* conformations. In the case of thymidine 4, the detection of both H1′–C6 and H1′–C2 strong correlations (Figs. 3B, 3D) suggests a motion around the glycosidic bond for a *syn/anti* equilibrium.

(b) In the second method, the H1′–(C6/8)–H6/8 connection is obtained by a single measurement, but without the benefit of chemical shift selection on carbon. In the proton–carbon–proton correlation (HCH) pulse sequence shown in Fig. 1D, the H1′ transverse magnetization created by the first 90° pulse is frequency labeled during the evolution delay t_1, and then transferred to C6/8 during a multiple-bond {^{1}H–^{13}C}-INEPT period τ. Other magnetization transfers are not discussed here for simplicity. During the following period τ' the antiphase coherence $(C6/8)_y(H1')_z$ is refocused with respect to H1′ and evolves simultaneously into an antiphase coherence with respect to H6/8, which is then refocused by the last one-bond {^{13}C−^{1}H}-INEPT into the inphase H6/8 magnetization for detection. Again, the use of 180° H1′-selective pulses suppresses the effect of homonuclear *J* modulation of

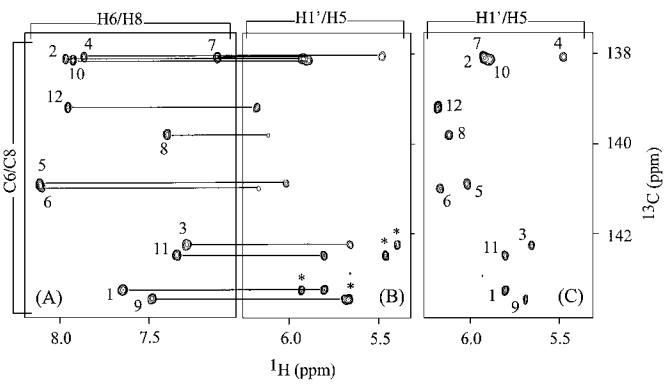

FIG. 2. Spectra of the DNA duplex [d(CGCGAATTCGCG)]$_2$ (strand concentration 5 mM; pH 7; temperature 35°C; 99.9% D_2O) recorded on a 500-MHz Varian Unity Inova spectrometer: (A) standard one-bond HSQC, (B) multiple-bond sHSQC (recorded by sequence 1B), and (C) multiple-bond sHMQC (recorded by sequence 1C). The spectra were recorded with spectral widths of 5 and 3 kHz, centered at 4.8 and 145 ppm, respectively in the ^{1}H and ^{13}C dimensions, 96 complex t_1 points, 512 complex t_2 points, repetition delay of 1.6 s. For the one-bond HSQC spectrum (A), 16 scans per FID, total measurement time of 1.5 h. For the multiple-bond correlation spectra (B, C), 256 scans per FID, total measurement time of 24 h. They are plotted at the same level. The cross peaks involving multiple-bond correlation within cytidine bases (^{1}H5–^{13}C6) are positive and labeled with an asterisk in (B), negative and not shown in (C).

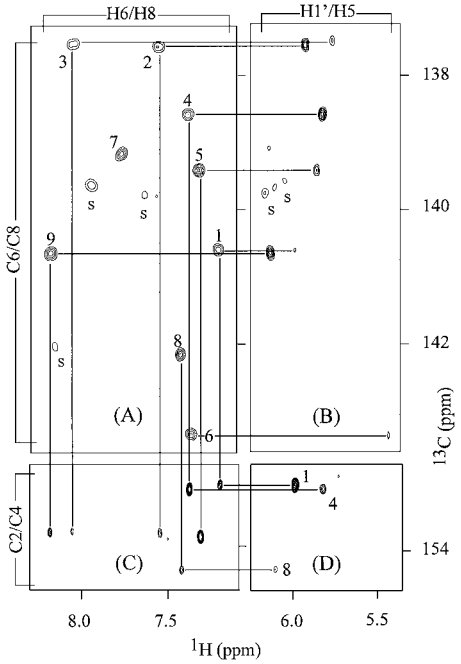

FIG. 3. Spectra of the dimeric G-quadruplex [d(GGGTTCAGG)]$_2$ (strand concentration 1.3 mM; pH 6.6; temperature 20°C; 99.9% D_2O) recorded on a 600-MHz Varian Unity Inova spectrometer: (A) standard one-bond HSQC, (B–D) multiple-bond sHMQC ($\tau = 55$ ms). The spectra were recorded with spectral widths of 6 and 4 kHz, centered at 4.8 and 145 ppm, respectively, in the ^{1}H and ^{13}C dimensions, 96 complex t_1 points, 512 complex t_2 points, repetition delay of 1 s. For the one-bond HSQC spectrum, 64 scans per FID, total measurement time of 3.5 h. For the multiple-bond correlation spectrum, 1024 scans per FID, total measurement time of 60 h. Only positive contours are shown. Cross peaks from a single-stranded form are labeled with a letter "s."

H1′ during long transfer delays. In another HCH version (Fig. 1E), homonuclear decoupling for H1′ during the first INEPT and t_1 periods is achieved with a 180° H2′/2″-selective pulse.

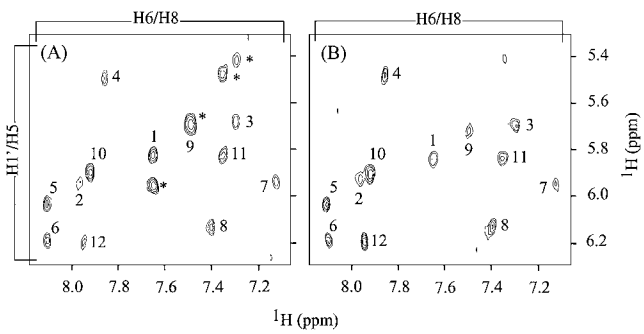

FIG. 4. HCH spectra of the DNA duplex [d(CGCGAATTCGCG)]$_2$ (same conditions as in Fig. 2): (A) recorded by sequence 1D and (B) recorded by sequence 1E. The spectra were recorded with spectral widths of 5 and 2 kHz, centered at 4.8 and 6.4 ppm, respectively, in the ω_2 and ω_1 dimensions, 36 complex t_1 points, 512 complex t_2 points, 1024 scans per FID, repetition delay of 1.6 s, total measurement time of 36 h. The intra-base H5–H6 cross peaks are labeled with an asterisk.

In the HCH spectra (Figs. 4A, 4B) all H1′–H6/8 cross peaks are observed. The intra-base H5–H6 cross peaks (Fig. 4A) are absent in the H2′/2″-selective version (Fig. 4B) due to unsuppressed $J_{H5,H6}$ modulation.

Direct H1′–H6/8 cross peaks and normally good resolution of both types of protons provide for the HCH experiments' simpler spectral interpretation than for the method (a), especially in case of unresolved C6/8, e.g., for nucleotides 2, 7, and 10 (Fig. 2). The HCH experiments can be extended to a 3D version, with the introduction of an evolution delay for the ^{13}C chemical shift (*20, 32*). However, the method (a) remains complementary since it is somewhat more sensitive and easier to set up. Furthermore, in case of *syn* glycosidic conformations the H1′ and H6/8 protons can also be sensitively correlated to C2/4 by multiple-bond ^{1}H–^{13}C correlation experiments.

High sensitivity and applicability at natural abundance are attractive qualities of the methods proposed here. The general difference as compared to earlier procedures (*19, 20*) is that we use fewer pulses. For instance, the sequence 1E uses only five ^{13}C pulses instead of seven in an equivalent experiment proposed previously (*20*). In case of multiple-bond correlation, both sequences proposed here avoid the effect of $J_{H,H}$ modulation on H1′ for evolution time t_1, in contrast to an earlier procedure for selective HMBC (*19*).

CONCLUSION

We have correlated sugar protons H1′ to base aromatic protons H6/8 via carbons C6/8 at natural abundance, using a combination of multiple-bond and one-bond J-couplings. The strategy can be used for other correlations in nucleic acids (*33*) (e.g., between sugar protons) and for other unlabeled molecules. The methods apply directly to conditions of weak enrichment, which are often used now to avoid undesirable couplings and relaxation of heteronuclei. Application to uniformly labeled nucleic acids would require selective ^{13}C pulses (*7*) and/or appropriate constant-time procedures (*32, 34*) in order to suppress unwanted coherence transfer pathways.

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