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Detection of Scalar Couplings Involving 2′-Hydroxyl Protons Across Hydrogen Bonds in a Frameshifting mRNA Pseudoknot

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Many important functional and structural features of RNA molecules critically rely on the 2′-OH hydroxyl protons, which distinguish RNA from DNA. The 2′-OH hydroxyl proton represents an excellent hydrogen bond (H-bond) donor and thus can stabilize tertiary interactions in RNA structures.[1,2] However, little detailed solution structural information is available on 2′-OH hydroxyl groups in RNA. This can mainly be attributed to rapid exchange with the solvent making the direct observation by NMR methods difficult.[3]

Here we present an NMR analysis of two slowly exchanging 2′-OH hydroxyl protons of the pea enation mosaic virus RNA1 (PEMV-1) pseudoknot.[4,5] This mRNA motif stimulates programmed −1 ribosomal P1−P2 frameshifting.[4] The 2′-OH hydroxyl protons of ribose sugars C15 ($\delta \approx 9.6$ ppm) and C16 ($\delta \approx 8.7$ ppm) function as H-bond donors, placing the adenosine bases A25-A27 of the PEMV-1 pseudoknot in the minor groove of stem S1 by formation of hydrogen bonds with N1 of A27 and N1 of A25, respectively (Figure 1), stabilizing[5] the stem S1 to loop L2 triple-strand interaction.[4] The chemical shifts of these two 2′-OH protons are shifted downfield relative to a uridine 2′-OH proton in a UNCG tetraloop ($\delta \approx 6.5-7.0$ ppm),[6,7] which donates a hydrogen bond to the guanosine O6 oxygen, but similar to the shift found for a 2′-OH to N hydrogen bond in an ATP-binding RNA aptamer.[8] Assignments of the 2′-OH proton resonances of C15 and C16 were obtained using a nonrefocused ^{1}H,^{13}C CPMG HSQC experiment (Supporting Information Figures 1A and 2), via detection of intraresidual three-bond ^{n}J(2′OH,C3′) scalar couplings,[9] confirming earlier findings from through-space NOE correlations.[4]

Here, we unambiguously verify the existence of a tertiary H-bonding interaction between a cytidine 2′-OH hydroxyl proton and an accepting N1 nitrogen of adenosine (Figure 1) via the detection of a measurable scalar J coupling across a hydrogen bond.[10−12] The sensitivity of detecting correlations involving exchangeable 2′-OH hydroxyl protons critically depends on efficient transfer of proton magnetization to long-range coupled nuclei without incurring severe losses due to chemical exchange. Magnetization in the presence of an exchange process characterized by the rate constant k_{ex} can be preserved by applying a Carr−Purcell−Meiboom−Gill (CPMG)[13] train of π-pulses, provided that $k_{ex}\cdot\tau(\text{cpmg}) \ll 1$.[14] We propose a nonrefocused CPMG HSQC[15], recording $2I_yS_z$ magnetization during t_2 to observe heteronuclear 2′-OH hydroxyl proton correlation spectra (Supporting Information Figure 1A). The observed signals are dispersive and antiphase with respect to the scalar J_{IS} coupling (Figure 1). Additionally, water-flipback shaped $\pi/2$ pulses keep the water magnetization along z to minimize further losses due to exchange of 2′-OH hydroxyl proton magnetization with water.[16]

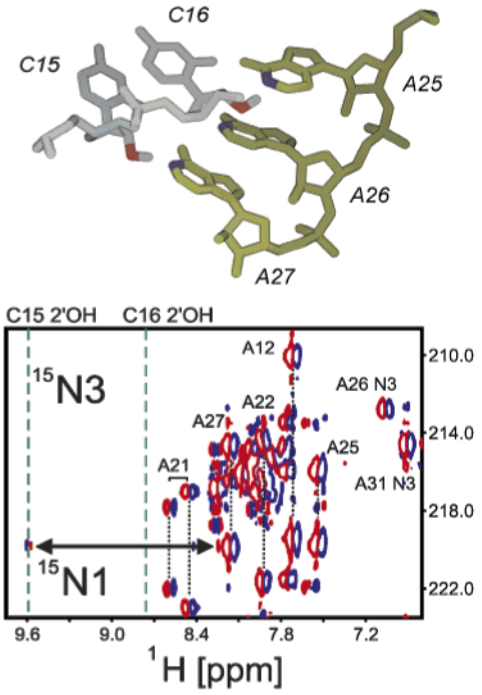

Figure 1. (Upper panel) Minimized average structure of the PEMV-1 pseudoknot mRNA showing details of tertiary interactions.[4] Loop L2 nucleotides A25, A26, and A27 stack traversing the minor groove. C15 and C16's 2′-OH hydroxyl protons donate hydrogen bonds to accepting N1 base nitrogen of A27 and A25, respectively. (Lower panel) Nonrefocused ^{1}H,^{15}N CPMG HSQC at 600 MHz (298 K) of uniformly ^{13}C,^{15}N-labeled 33-mer PEMV-1 RNA (~1 mM) showing a correlation of the 2′-OH hydroxyl proton of C15 to the N1 nitrogen resonance of A27 mediated by a cross hydrogen bond scalar coupling. A delay of $\Delta = 822$ μs ($n = 64$) was used, giving a total period of 57 ms for the antiphase buildup. 1472 transients were acquired for a total measurement time of 3.5 d. The ^{1}H carrier frequency was set to 4.76 ppm, and the ^{15}N carrier was set to 210.8 ppm. Assignments for observed intraresidual correlations within adenosine bases via ^{2}J(H2,N1/3) scalar couplings are given.[4] The corresponding correlation between the 2′-OH proton of C16 and the N1 of A25 is missing, likely due to unfavorable transverse relaxation properties of the proton. Attempts to perform the experiment at lower temperatures (278 K) to observe the missing C16 2′OH−A25 N1 correlation failed. Spectra were recorded on a four-channel Varian Inova 600 MHz spectrometer equipped with an actively shielded z-gradient triple-resonance probe. Spectra were processed and analyzed using FELIX 2000 (MSI, San Diego, CA) and NMRPipe program packages. The sample buffer contained 10 mM phosphate buffer, pH 6.0, 100 mM KCl and 5 mM $MgCl_2$ in 250 μL of 90% H_2O/10% D_2O.

The nonrefocused ^{1}H,^{15}N CPMG HSQC of uniformly ^{13}C,^{15}N-labeled 33-mer PEMV-1 RNA reveals a correlation of the 2′-OH hydroxyl proton of C15 to the N1 nitrogen resonance of A27 mediated by a scalar coupling across the hydrogen bond (Figure 1). Relaxation interference between the 2′OH hydroxyl proton

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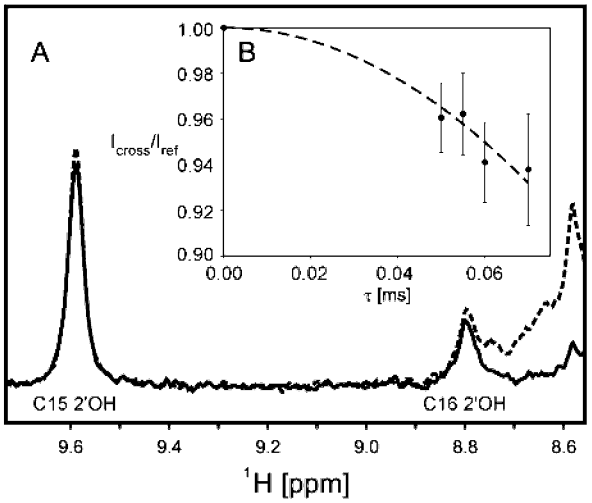

Figure 2. (A) Quantitative 1D {^{15}N} spin−echo difference experiment recorded with a constant dephasing period of $\tau = 50$ ms. Efficient water suppression combined with minimal baseline distortion was achieved by the excitation sculpting technique. Two experiments were recorded. In the first experiment, the ^{15}N π-pulse is applied at the end of the spin−echo period τ so that the observed 2′-OH hydroxyl protons are decoupled (dashed spectrum) yielding the reference integral. In the second (cross-) experiment, the ^{15}N π-pulse is shifted to the midpoint of the spin−echo delay, causing ^{1h}J(2′OH,N) dephasing and thus signal attenuation for the full period τ (solid spectrum). (B) I_{cross}/I_{ref} ratio for the C15 2′-OH hydroxyl proton to the A27 N1 nitrogen resonance obtained with varying spin−echo periods τ. Four different data sets were recorded with $\tau = 50$, 55, 60, and 70 ms, respectively. The dashed line shows the nonlinear fit to $\cos[\pi^{1h}J(2'\text{OH,N})\tau]$ ($R = 0.97$). Error bars were estimated based on the individual S/N ratios.

chemical shift anisotropy (CSA) and the corresponding ^{15}N1 CSA potentially provides an additional magnetization transfer mechanism. This unambiguously identifies the nitrogen acceptor resonance frequency. The similarity between the chemical shifts of N1 and N3 nitrogens of A27 and A25 is striking, providing indirect evidence that the N1 resonance of A25 functions as a similar H-bond acceptor. Highfield-shifted adenosine nitrogen resonances were also indicative of a similar 2′-OH-nitrogen H-bonding interaction in an ATP-binding RNA aptamer.[8]

A quantitative 1D {^{15}N} spin−echo difference experiment where two interleaved 1D experiments are recorded (Supporting Information Figure 1B) was chosen to measure couplings between 2′-OH hydroxyl protons and the accepting N1 base nitrogens.[17] The ^{1h}J(2′OH,N) couplings are calculated from the ratio of measured integrals according to $^{1h}J(2'\text{OH,N}) = \cos^{-1}(I_{cross}/I_{ref})/(\pi\tau)$. The ^{1h}J(2′OH,N) cross hydrogen bond scalar coupling for the C15/A27 interaction is 1.7 ± 0.1 Hz (Figure 2). The quality of the nonlinear fit of the ratio of measured integrals to $\cos[\pi^{1h}J(2'\text{OH,N})\tau]$ as a function of the dephasing period is good in case of the C15/A27 interaction ($R = 0.97$). The ^{1h}J(2′OH,N) cross hydrogen bond scalar coupling for the C16/A25 appears to be larger, 3.5 ± 0.3 Hz, although the corresponding direct correlation between the H-bonded 2′-OH hydroxyl proton of C16 and the N1 of A27 is missing. The quality of the fit in this case is worse, $R = 0.81$, reflecting the poor S/N ratio of the C16 2′-OH hydroxyl proton, and thus the size of the coupling should be interpreted with care.

To the best of our knowledge, cross hydrogen bond scalar couplings of the type O−H···N have not been observed previously. However, ^{1h}J(H,N) cross hydrogen bond scalar coupling constants of the type N−H···N in canonical- and noncanonical base pairs range from approximately 2 to 4 Hz[12] while ^{2h}J(H,P) couplings between exchangeable hydroxyl protons of serines and threonines and the FMN 5′-phosphate group bound to *Desulfovibrio vulgaris* flavodoxin, O−H···O−P, appear to be smaller in size, ranging from 0.6 to 1.7 Hz.[18]

The H-bonding properties of two slowly exchanging 2′-OH hydroxyl protons of the PEMV-1 mRNA pseudoknot are investigated by high-resolution NMR. For the first time, we present unequivocal evidence for the interresidual tertiary H-bonding interaction between two 2′-OH hydroxyl protons located in stem S1 and the accepting adenine N1 base nitrogen in loop L2 in a frameshifting mRNA motif. The observed ^{1h}J(2′OH,N) cross hydrogen bond scalar couplings for the C15/A27 and C16/A25 interactions are in perfect agreement with our previous NOE-based solution structure.[4] Our findings reveal a detailed picture of critical noncanonical interactions of loop−stem interactions of an RNA pseudoknot. Furthermore, the tailored techniques presented in this study can facilitate other NMR investigations hampered by unfavorable solvent exchange properties of involved proton nuclei.

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Supporting Information Available: Two Figures, showing the pulse sequences and a nonrefocused ^{1}H,^{13}C CPMG HSQC spectrum (PDF). This material is available free of charge via the Internet at http://pubs.jacs.org.

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