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Communications

Use of a Pyrimidine-Filtered Pulse Sequence Simplifies the Proton-NMR Spectrum of RNA

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RNA plays important roles in biological processes, including propagation of genetic information and enzymatic catalysis.[1] Two-dimensional ^{1}H-NMR spectroscopy can provide a wealth of structural information about RNA molecules. As the first step in these structural studies, complete spectral assignments must be made. Assigning base protons and sugar H1' protons is often straightforward in helical regions.[2] However, assignments are difficult in the presence of mismatches and in single-stranded regions.[3,4] The combination of ^{13}C-^{1}H- and ^{1}H-^{1}H-correlated experiments and NOE experiments enable the identification of each individual spin system. Identifying sequential base-H1 and base-sugar cross-peaks in NOESY spectra is important because it provides the most useful information for making these assignments. However, spectral overlap in these regions often limits researchers' ability to make assignments in RNA oligonucleotides, especially those that are large.

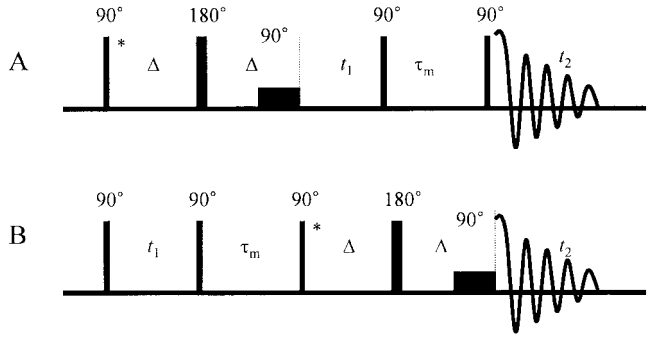


Figure 1. Pulse sequences for pyrimidine-filtered experiments, which eliminate H6 magnetization in the ω_1 dimension (A) or in the ω_2 dimension (B) in NOESY spectra. The wide bar represents the shaped pulse in the H1'/H5 region. The phase of the shaped pulse is equal to that of the marked 90° pulse (asterisk), and the rest of phase cycle is fully analogous to that of the unfiltered experiments.

Sklenar and Feigon introduced the HOENOE experiment to select cytosine H6 doublets.[5] The HAL experiment was proposed to select singlets instead of doublets by Kojima and Kyogoku.[6] The HOENOE and HAL experiments were applied to simplify the proton NMR spectra of the DNA base-proton region. However, the HOENOE experiment suffers from the low signal intensities and experimental difficulties. The HAL experiment showed incomplete selection of the signals due to imperfect signal canceling. Here we present an experimental technique that can selectively filter the NOE connectivities involving pyrimidine H6 resonances to simplify the NOESY spectra of RNA oligonucleotides. Figure 1 shows the pulse sequences that have been employed in this work. After the Hahn-echo (90-Δ-180-Δ), the selective 90° pulse on H1'/H5 region causes elimination of pyrimidine H6 proton resonances. The ^{1}H 90° pulse, followed by a delay, $2\Delta=1/(2J_{H5\text{-}H6})$, produces an anti-phase ^{1}H doublet. Application of a selective θ pulse to H5 creates unobservable multiple-quantum coherence. For $\theta=90°$, all H5-H6 coherence is multiple-quantum, and therefore no signal is observed. On the other hand, for purine H8/H2, in-phase coherence is created by the 180° pulse between the delays. The use of selective pulse instead of non-selective spin lock pulse in the HAL experiment reduces the residual doublet peaks due to incomplete selection of the signals by phase cycling. The resulting two-dimensional spectra obtained are

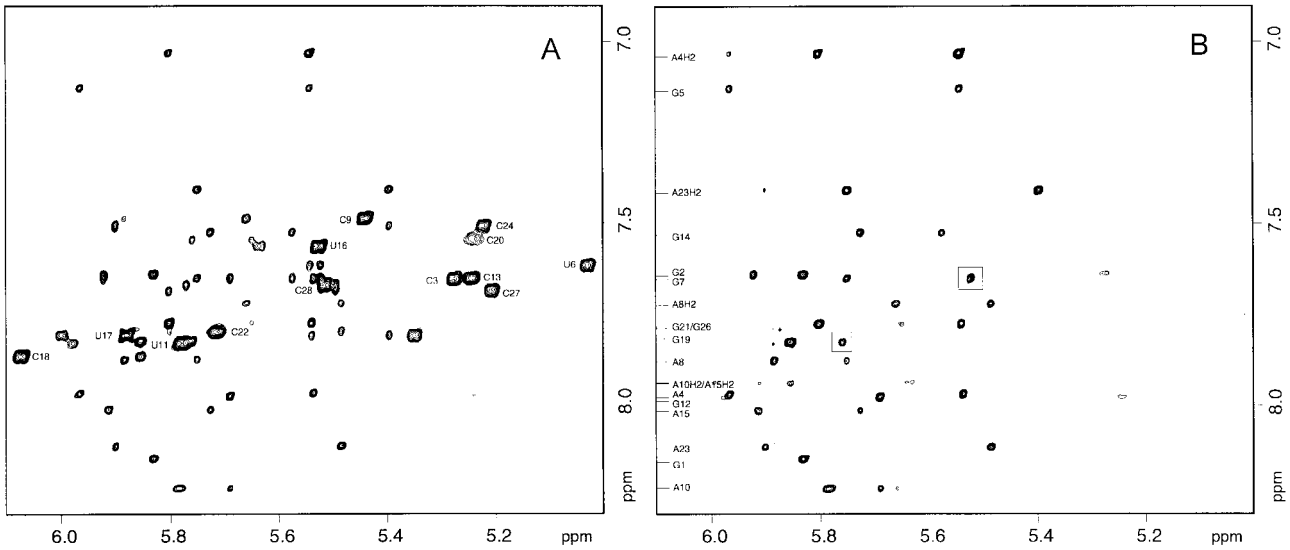
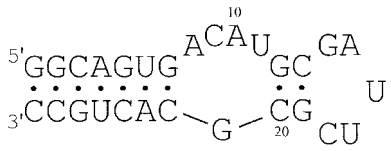


Figure 2. Expanded region of the 600 MHz ^{1}H-NMR spectra of a 28-nucleotide RNA molecule. In contrast to a standard NOESY experiment (A), a pyrimidine-filtered NOESY experiment (B) does not show NOEs involving pyrimidine H6 resonances. The pyrimidine crosspeak (H5-H6) (A) and purine resonances (H8/H2) assignments are labeled. The sample contained 1.5 mM RNA in 10 mM sodium phosphate at pH 6.5. A sinc3 shaped pulse centered at 5.7 ppm with 6 ms duration was used for pyrimidine H5 excitation. Data were acquired with 512×2048 complex points with 16 transients per increment. Mixing time was set to 400 ms. The boxed peaks that are difficult to identify in (A) due to spectral overlap are easily detected in (B).



Scheme 1. Secondary structure and numbering of the RNA.

nonsymmetrical because of the use of both selective filtration in one dimension and nonselective excitation in the other dimension.

Application of this pyrimidine-filtering technique to a 28-nucleotide RNA is illustrated in Figure 2. This RNA, r(GGCAGUGACA UGCGAUUCGC GCACUGCC), is the binding site for the *Saccharomyces cerevisiae* virus[7] shown in Scheme 1 and forms a hairpin-stem-loop-stem structure. A detailed study of this RNA will be presented elsewhere. The base-H1' crosspeak region from a standard NOESY experiment is shown in Figure 2A. Figure 2B shows the same region from a pyrimidine-filtered NOESY experiment. Crosspeaks in this region do not show NOEs involving H6 resonances. Some of H8-H1' crosspeaks that cannot be seen in a standard NOESY spectrum due to spectral overlap with intense H5-H6 crosspeaks can be observed in a pyrimidine-filtered NOESY spectrum (Figure 2B).

In this study, we have presented pyrimidine-filtered pulse sequences that can be used to identify the sequential base-ribose NOE crosspeaks in RNA. This technique can separate the crosspeaks where overlap of purine and pyrimidine occurs. The experiments also can be used to selectively filter H5 resonances if H1'/H5 overlap occurs. Presently, there are various NMR pulse sequences[8] which can provide assignment tools for ^{13}C/^{15}N-labeled RNAs. However, the experiments presented here do not require isotope labeling or any special hardware for hetero-nuclear experiments. NMR experiments using pyrimidine-filtered pulse sequences have no magnetization transfer steps, but they do require delays for multiple-quantum generation by homo-nuclear coupling ($J_{H5\text{-}H6} \sim 7.5$ Hz). Compared to a conventional unfiltered technique, this technique does suffer from potential additional signal losses of [1-exp ($-2\Delta/T_2$)]. In our case, T_2 for purine H8 protons ranges from 120 to 60 ms, so 40 to 70%, on average 52%, signal losses are observed. Pulse sequences also can be used for studies of DNA, which is not commonly studied by isotope labeling.

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