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# Assignment of NH resonances in nucleic acids using natural abundance $^{15}N$-$^{1}H$ correlation spectroscopy with spin-echo and gradient pulses

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It is well known that 2D $^{15}N$-$^{1}H$ correlation spectra can resolve overlapped imino proton resonances in the downfield NMR spectra of nucleic acids according to their $^{15}N$ chemical shifts, and that these resonances can be assigned by base type on that basis, independent of conformation. This information can be extremely important in determining the solution structure of a nucleic acid by NMR, but previously could only be obtained using $^{15}N$-labeled, or very concentrated samples. Here we report the design of a gradient-enhanced, jump-return spin echo version of an $^{15}N$-$^{1}H$ HMQC experiment (GE-JRSE HMQC) that is sensitive enough to work on unlabeled nucleic acid samples at normal NMR concentrations. This experiment has led to the assignment of imino proton resonances with non-Watson–Crick chemical shifts in the spectrum of a 29 residue oligoribonucleotide that models the sarcin/ricin loop from 28S rRNA.

Gradient-enhanced NMR; $^{15}N$ NMR; HMQC; Solvent suppression; Ribosomal RNA; Sarcin/ricin loop

## 1. INTRODUCTION

Determination of the internucleotide hydrogen bonding pattern of a nucleic acid is an essential step in solving its solution structure by NMR. Nucleic acids that contain stable hydrogen-bonded structures invariably have resonances in the downfield region of their proton spectra, between 10 and 15 ppm [1]. Exposed imino protons belonging to free thymine, uracil and guanine nucleotides exchange too rapidly with solvent to be observed at physiological pH and temperatures. However, when these imino protons are hydrogen bonded or otherwise protected from solvent exchange, their resonances are visible in $^{1}H$ NMR spectra in the downfield region. Assignment of these resonances is a critical step in the determination of the secondary structure of a nucleic acid by NMR [2,3].

$^{15}N$-$^{1}H$ correlation experiments are particularly helpful for assigning imino proton resonances that have unusual chemical shifts because uracil (or thymine) N3 and guanine N1 $^{15}N$ chemical shifts are separated by approximately 15 ppm, independent of the conformation of the nucleic acid [4]. Several groups have used a variety of different types of heteronuclear correlation experiments to harvest the information that $^{15}N$-$^{1}H$ correlations can provide [5–7]. Since the natural abundance of $^{15}N$ is only 0.37%, $\gamma_H/\gamma_N = 9.88$, and oligonucleotide samples generally are available only in millimolar concentrations, nearly every $^{15}N$-$^{1}H$ correlation experiment performed on nucleic acids to date has depended on the synthesis of an $^{15}N$-labeled sample. In a few cases where sample concentrations exceeded 12 mM, $^{15}N$-$^{1}H$ correlation spectra have been obtained for DNA oligonucleotides at natural abundance [8,9]. We describe here an experiment that has enabled us to acquire a natural abundance, proton observed, $^{15}N$-$^{1}H$ HMQC spectrum for a 3 mM sample of a 29-nucleotide RNA. This experiment allows us to assign its imino proton resonances according to base type.

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## 2. MATERIALS AND METHODS

The 3 mM sample of a 29-nucleotide RNA which models the sarcin/ricin loop from rat 28S rRNA used in this study was prepared as described previously [10].

The NMR experiment is illustrated in Fig. 1. A normal HMQC pulse sequence [11] has been modified to include jump-return, spin echo proton pulses for the suppression of unwanted signals arising from water [12,13]. To suppress the residual water signal further, pulsed gradients of equivalent intensity are applied along all three axes during both the $(2J)^{-1}$ delays to dephase $^{1}H_2O$ magnetization. The combined jump-return and gradient spin echo pulse sequence suppresses the water signal by approximately $10^6$. Quadrature detection in $t1$ is obtained using the States-TPPI method [14].

## 3. RESULTS AND DISCUSSION

Several gradient-enhanced, heteronuclear correlation experiments have been reported in the literature [15,16]. These experiments have employed pulsed gradients for coherence order pathway selection, which eliminates the need for phase cycling, and for suppressing $^{1}H_2O$ magnetization; reducing acquisition time in the bargain. In our hands, these experiments were not as sensitive as the

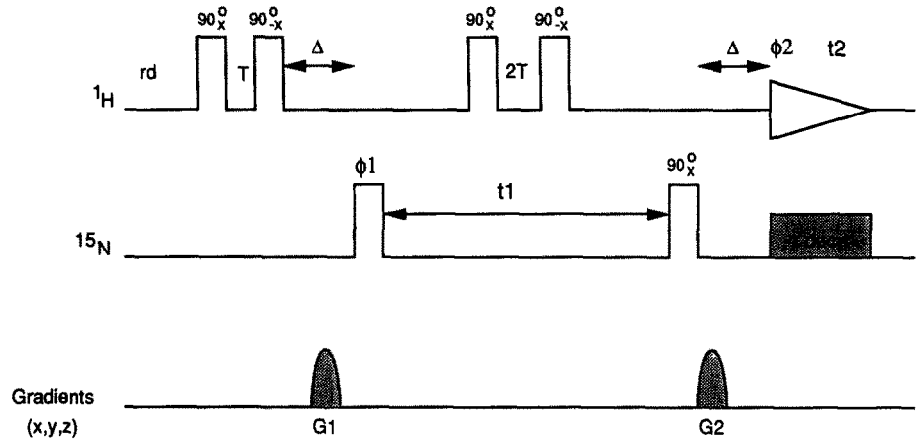


Fig. 1. Pulse sequence for the gradient-enhanced jump-return spin echo HMQC. For water suppression the proton pulses have been replaced with a jump-return spin echo sequence [12,13]. In addition gradient pulses along all three axes are applied prior to the first, and just after the second carbon pulse. The symbols are defined as follows: *rd* denotes the relaxation delay, $T = (4 \times \Delta\nu)^{-1}$ where $\Delta\nu$ is the distance between the maximum of proton excitation and the carrier frequency, $\Delta = [2J(^{15}N\text{-}^{1}H)]^{-1}$, and dec represents GARP decoupling at low power [21]. The phases of $\Phi_1$ and $\Phi_2$ alternate $x_1 - x$ on successive scans. Quadrature detection is achieved using the states–TPPI method [14].

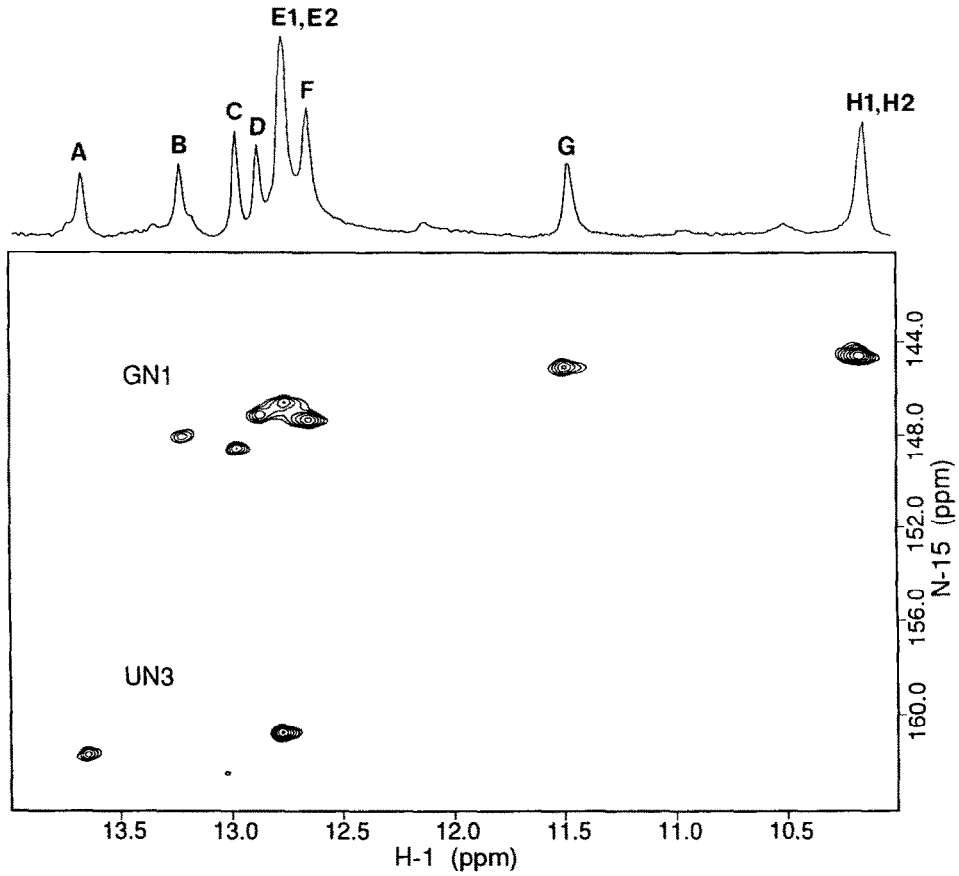


Fig. 2. 2D natural abundance $^{15}N$-$^{1}H$ spectrum of the sarcin/ricin loop from rat 28S rRNA in 95/5 (v/v) $H_2O/D_2O$. The imino proton-nitrogen region is illustrated here, with an imino proton spectrum acquired with a 1D jump-return spin echo sequence plotted along the proton axis. Imino proton peaks are labeled A through H2. The 3 mM RNA sample was dissolved in 50 mM KCl, 15 mM NaCl, 0.5 mM EDTA, 10 mM phosphate buffer pH 7.6, $T = 10°C$. The experiment was carried out on a GE-Omega 500 MHz PSG spectrometer equipped with an S-17 shielded gradient probe. The proton spectral width was 10,000 Hz with the carrier on water (4.9 ppm, referenced using an internal dioxane chemical shift standard, 3.741 ppm), while the nitrogen spectral width was 1200 Hz, centered at 158 ppm. Ninety degree pulses for the proton and nitrogen frequencies were 9 $\mu$s and 55 $\mu$s, respectively. The initial evolution delay was optimized to invert aliased resonances after 180° linear phase correction [22]. 640 FIDs were accumulated for each of 120 $t1$ experiments, giving a total experiment time of 36 h. The pulse train delays were set as follows: $T = 68$ $\mu$s, $\Delta = 5.3$ ms, rd = 1.8 s. GARP decoupling with low power $^{15}N$ pulses (1.2 kHz) was applied during acquisition. Gradient pulses $G1$ and $G2$ were equivalent ($B/x = 7$ G/cm in each direction). In $f2$ 10 Hz line broadening was applied while in $f1$ a 90° shifted squared sine bell was used for apodization. In order to produce the final spectrum the 2048 × 256 matrix was circularly shifted downfield by 64 points in the nitrogen dimension and both positive and negative contours plotted. All processing was carried out on a Silicon Graphics IRIS 4D-25 workstation using Felix 2.0 (Hare Research).

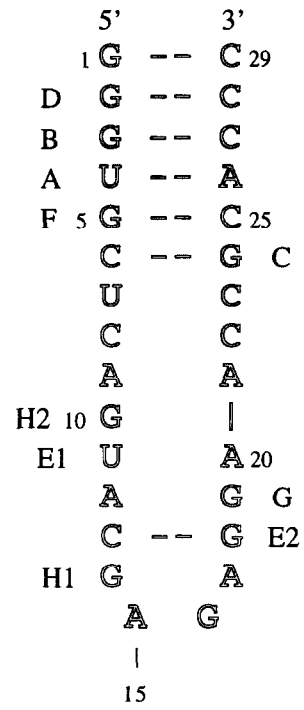


Fig. 3. The sequence and secondary structure of E73 RNA. Shown here is the sequence of E73 RNA with the imino protons that are observed at 10°C labeled A through H2. Watson–Crick base pairing is denoted by dashed lines. One-dimensional NOE difference spectra and 2D NOESY spectra collected for E73 show two sets of sequential NOE correlations: D-B-A-F-C and H2-E1-G-E2-H1 [10] (A.A.S. and P.B.M., unpublished data). The $^{1}H$-$^{15}N$ correlation spectrum shown in Fig. 2 identifies protons A and E1 as belonging to uracil residues; the other observed imino protons belong to guanine residues. With this information, H2-E1-G-E2-H1 can be assigned to residues G10-U11-G19-G18-G14 (see Table I).

GE-JRSE HMQC experiment when applied to $^{15}N$-labeled RNA. The loss in sensitivity from the use of gradients for coherence order selection was greater than $1/\sqrt{2}$ loss expected due to selection of the absolute sign of coherence. The GE-JRSE HMQC experiment, on the other hand, delivered outstanding sensitivity when applied to $^{15}N$-labeled RNA. High resolution 2D $^{15}N$-$^{1}H$ spectra could be collected on 2 mM $^{15}N$-pDG07 RNA (a 64-nucleotide deletion mutant of *E. coli* 5S RNA, [17]) samples in under 15 min (data not shown).

Table I

Imino proton and nitrogen chemical shift assignments for E73 RNA. the E73 imino proton peaks observed at 10°C are labeled A through H2 in the first column, with their proton chemical shifts given in the second column. The third column lists the corresponding nitrogen chemical shifts for these NH groups. E73 residues are numbered as shown in Fig. 3.

| | Chemical shift (ppm) | | |
|---|---|---|---|
| Imino proton | H-1 | N-15 | Residue |
| A | 13.65 | 161.7 | U4 |
| B | 13.22 | 148.1 | G3 |
| C | 12.97 | 148.6 | G24 |
| D | 12.87 | 147.1 | G2 |
| E1 | 12.77 | 160.8 | U11 |
| E2 | 12.76 | 146.6 | G18 |
| F | 12.65 | 147.4 | G5 |
| G | 11.50 | 145.1 | G19 |
| H1 | 10.17 | 144.7 | G14 |
| H2 | 10.17 | 144.7 | G10 |

Fig. 2 shows the 2D $^{15}N$-decoupled, natural abundance $^{15}N$-$^{1}H$ HMQC (GE-JRSE HMQC) spectrum given by 'E73', a 29-nucleotide RNA which corresponds to the sarcin/ricin loop from rat 28S rRNA [10, 18–20] (see Fig. 3). The region containing imino proton-nitrogen crosspeaks has been plotted with a 1D imino spectrum drawn along the proton axis for comparison. The uracil H3-N3 crosspeaks occur at the bottom of the spectrum at a nitrogen chemical shift of about 161 ppm, with the guanine H1-N1 crosspeaks aligned above them. The two groups of crosspeaks are separated by about 15 ppm in the nitrogen dimension. All but two of the ten imino resonances visible in the normal exchangeable $^{1}H$ spectrum are completely resolved in the 2D HMQC spectrum. An undecoupled version of this experiment might be used to extract $^{15}N$-$^{1}H$ coupling constants.

Earlier exchangeable proton spectra of E73 revealed two sets of imino proton peaks with sequential NOE connectivities. One set included a downfield-shifted uracil NH which gave a strong NOE to an adenine H2 proton, and was quickly assigned to residues G2-G3-U4-G5-G24 [10]. Unfortunately, it was unclear which, if any, of the remaining peaks belonged to uracil residues, especially given their upfield chemical shifts. Nor was it entirely certain that peaks near 10 ppm were not downfield-shifted amino resonances. However, in conjunction with the experiment described here, the other set of NOEs can be unambiguously assigned to residues G10-U11-G19-G18-G14 (see Fig. 3 and Table I). Thus the gradient-enhanced jump-return spin echo $^{15}N$-$^{1}H$ HMQC experiment described here provides a simple means to assign imino resonances in nucleic acid proton spectra according to base type, and is sensitive enough so that unlabeled samples of the concentrations normally used for $^{1}H$ spectroscopy can be used. While this experiment has been applied here to an RNA only 29 nucleotides long, in principle it can provide useful information for considerably larger nucleic acids.

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