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Measurement of H2′-C2′ and H3′-C3′ dipolar couplings in RNA molecules

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Abstract

The quality of nucleic acid solution structures can be significantly improved using residual dipolar coupling data. However, many of the one-bond couplings that could be used for this purpose are difficult to measure. Conventional 2D experiments are often unable to reveal one-bond H2′-C2′ and H3′-C3′ couplings in large RNA molecules due to spectral overlap. Here we show how to use 3D HCcH-COSY and Relay HCcH-COSY to measure one-bond H2′-C2′ and H3′-C3′ couplings which improved the precision of the structures obtained recently for a 42 nucleotide RNA.

Since 1990, solution structures have been obtained for many RNA oligonucleotides by NMR using J-coupling and NOE data (Moore, 1995; Pardi, 1995; Varani et al., 1996). On the whole, however, RNA structures are not well determined by such data both because RNA is proton-poor compared to proteins, for example, and because the overlap of ribose proton resonances makes it difficult to fully exploit the few it has. In addition, many RNAs have elongated structures, and the overall shapes of elongated molecules are hard to constrain using local torsional information and short range distances.

Residual dipolar coupling data are extremely interesting in this regard. They can provide information about the orientations of specific chemical bonds throughout a nucleic acid with respect to a single coordinate frame (Prestegard et al., 2000; Bax et al., 2001; de Alba and Tjandra, 2002), and thus help determine its overall shape, even if it is elongated (Tjandra et al., 2000; Vermeulen et al., 2000; Warren and Moore, 2001; Sibille et al., 2001). Unfortunately dipolar couplings can be measured only for a small number of C-H bonds in a large RNA using 2D ^{1}H-^{13}C correlation experiments (Tjandra and Bax, 1997; Brutscher et al., 1998; Tian et al., 2001) because only a limited number are associated with well resolved resonances. Only the C2-H2 (in adenines), C5-H5 and C6-H6 (in pyrimidines), C8-H8 (in purines) and H1′-C1′ couplings tend to be readily measurable. The resonances associated with H2′-C2′ and H3′-C3′ bonds, which could contribute valuble information are usually severely overlapped.

Here we show how the excellent dispersion of the H1′ and C1′ resonances can be exploited to measure H2′-C2′ and H3′-C3′ couplings. H2′-C2′ couplings can be measured using modified versions of the 3D HCcH COSY experiment (Hoogstraten and Pardi, 1998), and the H3′-C3′ couplings can be measured using a modified 3D relay HCcH COSY experiment (Pardi and Nikonowicz, 1992).

H2′ and H3′ resonances in RNA are routinely assigned using 3D HCcH-COSY and Relay-HCcH COSY experiments that correlate them to the H1′/C1′ resonances of the same ribose, which are usually easy to assign (Pardi, 1995). The experiment used here to measure H2′-C2′ couplings (Figure 1) is similar to the spin-echo difference constant-time HCcH-COSY experiment proposed by Hoogstraten and Pardi (Hoogstraten and Pardi, 1998). The version of that experiment that we have used to measure the H3′-C3′ couplings includes an extra delay to transfer magnetization from C2′ to C3′ (Figure 1). The magnetization

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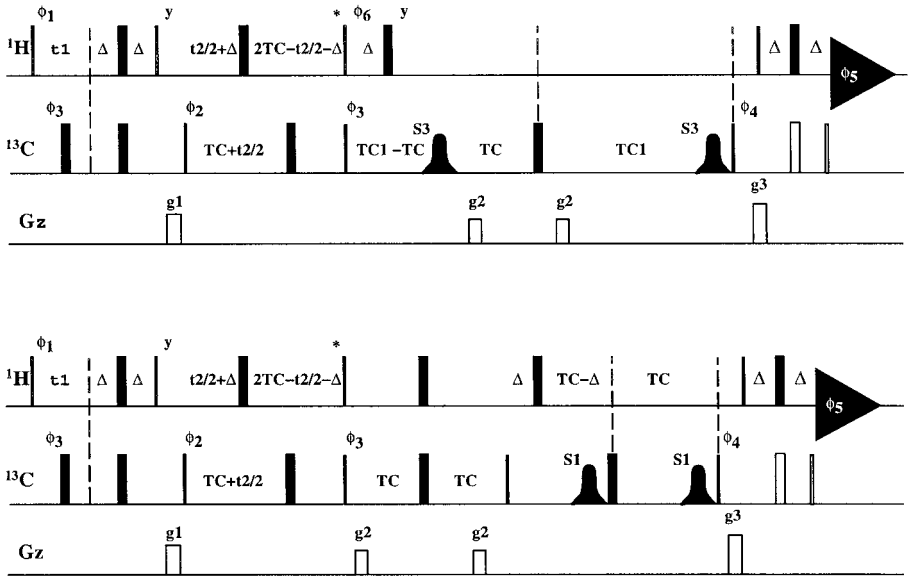

Figure 1. The top pulse sequence is that of the IPAP-HCcH-COSY experiment used to measure H2′-C2′ couplings, and the IPAP-relay-HCcH-COSY pulse sequence at the bottom is used to measure H3′-C3′ couplings. Here Δ = 1.5 ms, TC = 5.75 ms, TC1 = 11.75 ms, S1 and S3 are selective IBURP1 (Geen and Freeman, 1991) pulses of length 4.2 ms and 2.9 ms applied at 83.3 ppm and 91.9 ppm, respectively, used to invert C4′ and C1′ nuclei respectively. All the pulses have x phase unless specified. The ^{1}H transmitter was placed on the water signal at 4.75 ppm and the ^{13}C transmitter was placed at 86.3 ppm. Z Gradients g1 = 15 G cm^{-1}, g2 = 10.5 G cm^{-1} and g3 = 2400 G cm^{-1} were applied for 1 ms each. The sweep width in the direct dimension was 6000 Hz, 3375 Hz in the indirect carbon dimension and 1800 Hz in the indirect proton dimension. Complex data is acquired by incrementing ϕ_1 and ϕ_2 in a States-TPPI manner (Marion et al., 1989). Two experiments are recorded in an interleaved fashion, one with (IP) and one without (AP) the last two carbon pulses, which are shown as open boxes. In both the pulse sequences the proton pulses indicated by the asterix are useful in reducing the residual HDO signal. Phases: ϕ_1 = x, $\overline{x}$; ϕ_2 = x, x, $\overline{x}$, $\overline{x}$; ϕ_3 = x, x, x, x, $\overline{x}$, $\overline{x}$, $\overline{x}$, $\overline{x}$; ϕ_4 = x, $\overline{x}$, x, $\overline{x}$, $\overline{x}$, x, $\overline{x}$, x; ϕ_6 = y, $\overline{y}$, y, $\overline{y}$, $\overline{y}$, y, $\overline{y}$, y; for the IP experiment ϕ_5 = x, x, $\overline{x}$, $\overline{x}$, $\overline{x}$, $\overline{x}$, x, x; and for the AP experiment ϕ_5 = y, y, $\overline{y}$, $\overline{y}$, $\overline{y}$, $\overline{y}$, y, y.

transfer pathways for the two experiments are summarized below:

HCcH-COSY:

$$\mathrm{H1'(t1)} \xrightarrow{^1J_{H1'C1'}} \mathrm{C1'(t2)} \xrightarrow{^1J_{C1'C2'}} \mathrm{C2'} \xrightarrow{^1J_{H2'C2'}} \mathrm{H2'(t3).}$$

Relay HCcH-COSY:

$$\mathrm{H1'(t1)} \xrightarrow{^1J_{H1'C1'}} \mathrm{C1'(t2)} \xrightarrow{^1J_{C1'C2'}} \mathrm{C2'} \xrightarrow{^1J_{C2'C3'}} \mathrm{C3'} \xrightarrow{^1J_{H3'C3'}} \mathrm{H3'(t3).}$$

FIDs are acquired without decoupling, and H2′-C2′ and H3′-C3′ couplings of interest result in the splitting of H2′ and H3′ resonances. When spectra are recorded without decoupling the number of crosspeaks doubles, of course, and this is likely to cause overlap. This problem can be minimized using the IPAP methodology of Ottiger et al. (1998) during the last reverse INEPT pulse sequence. This results in two spectra, each containing only one component of every doublet. At each time point, two experiments are carried out, one with and one without the last two carbon pulses (Figure 1), and the resulting FIDs are stored separately. When the last two carbon pulses are used in the relay HCcH-COSY experiment, the magnetization at the end of the pulse sequence is the inphase (IP) component, $H3'_x$, and without the last two pulses it is the antiphase (AP) component, $2H3'_xC3'_z$. When experiments are added the result is $H3'_x + 2H3'_xC3'_z$, which corresponds to one component of a doublet, while their subtraction gives $H3'_x - 2H3'_xC3'_z$ which corresponds to the other component of the doublet (Figure 2). Thus provided H1′, C1′ and H2′/H3′ assignments are available, H2′-C2′/H3′-C3′ dipolar couplings can be measured, and that is useful because C2′ and C3′ resonances are often difficult to assign.

The experiments described above have been applied to a 42 nucleotide RNA called SPIN2 which contains the Helix IV/V arm from *Spinacia oleracea* (spinach) chloroplast 5S rRNA. Two 175 µl ^2H_2O samples in 50 mM NaCl, 0.1 mM EDTA and

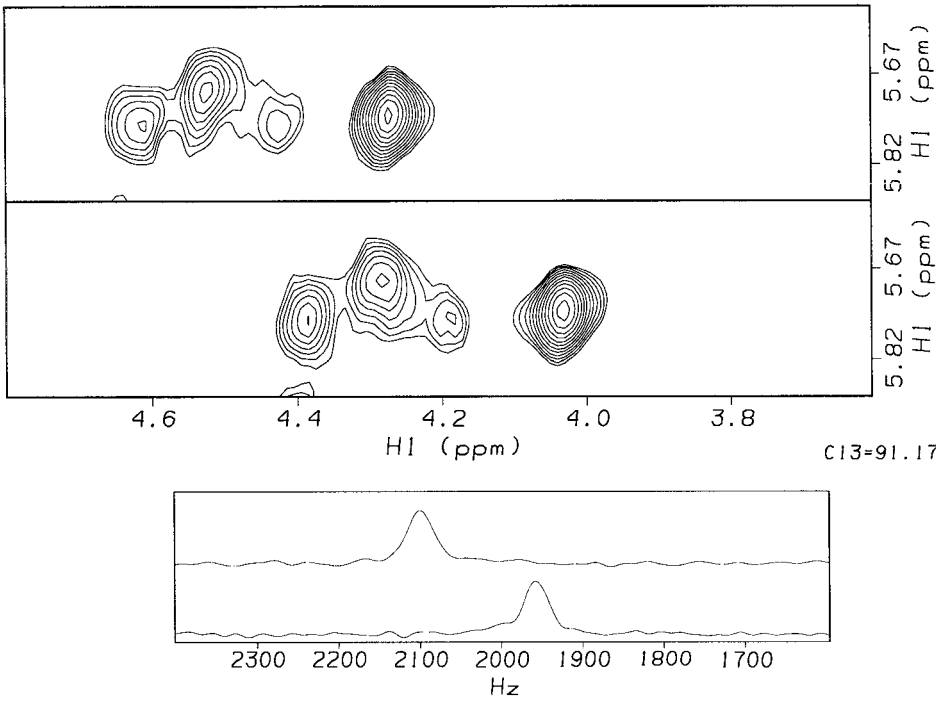

Figure 2. Measurement of H3′-C3′ J coupling in an unoriented sample at 25 °C using the IPAP-Relay HCcH-COSY. This experiment correlates H3′ to H1′/C1′ resonances of the same ribose sugar. Each strip contains one of the two components of the H3′ doublet. The difference in chemical shift between the two spectra is due the one-bond J coupling between the H3′ and C3′ nuclei. The lower panel shows 1D traces along the H3′ dimension for G84, for which the H3′-C3′ J coupling is 140.3 Hz. Spectra were acquired with 44* × 37* × 384* points which corresponds to 23.8 ms in t1, 10.96 ms in t2 and 60 ms in t3. Data in the t2 (^{13}C) dimension was linear predicted to double the amount of data. The data were apodized by a 72° shifted sine function in t1 and 90° shifted sine function in t2 and 90° shifted sine squared function in t3. and zero filled to give 256 × 256 × 256 matrices. Data in the t3 dimension was zero filled to extract only resonances between 6.1 and 3.45 ppm. Eight scans were recorded for each FID, leading to an acquisition time of about 72 h.

2.5 mM cacodylate pH 6.0 were used for measurements. The unoriented sample contained 1.3 mM ^{13}C/^{15}N SPIN2 and the oriented sample contained 1.6 mM ^{13}C/^{15}N SPIN2 and 8 mg/ml Pf1 phage (Hansen et al., 1998). The spectra were processed using FELIX 97 (MSI/BIOSYM, INC) and peaks were picked using SPARKY (Goddard and Kneller, 2001). All experiments were performed on an 600 MHz Varian Unity Plus spectrometer which was Z gradient-capable.

These experiments are quite sensitive, and using them we were able to measure 20 H2′-C2′ and 27 H3′-C3′ dipolar couplings in a 42 nucleotide RNA. Only 3 H2′-C2′ and 4 H3′-C3′ couplings could be measured in this molecule using 2D ^{1}H-^{13}C correlation experiments. The H2′-C2′ and H3′-C3′ dipolar couplings observed varied from +31.9 to −32.3 Hz, and based on replicate measurements, the RMS error was 2.7 Hz for the H2′-C2′ couplings and 2.6 Hz for the H3′-C3′ couplings.

The impact of including the H2′-C2′and H3′-C3′ dipolar coupling data on an RNA structure determination can be considerable. The RMSD from the mean for a family of ten structures of SPIN2 calculated using 284 distance restraints and 311 torsion angle restraints was 2.65±1.12 Å (P. Vallurupalli and P.B. Moore, *in preparation*). Addition of 59 H8-C8, H6-C6, H5-C5, H2-C2 and H1′-C1′ dipolar couplings reduced the RMSD for the family to 1.16±0.5 Å. Adding 30 H2′-C2′ and H3′-C3′ dipolar couplings measured using three dimensional experiments described above resulted in a additional improvement in the precision of the family structures. The RMSD was reduced to 0.97±0.4 Å. (In all the structure calculations dipolar couplings from sugars undergoing conformational exchange were not used.)

Using the experiments described above H2′-C2′ and H3′-C3′ couplings can be measured in RNAs of substantial size. In the case of SPIN2, the use of these experiments brought about a 50% increase in the number of dipolar couplings available to con-

strain subsequent structure calculations, and their use improved the precision of the calculated structures. These experiments do not require assignments for C2′ and C3′ resonances, which may not be readily available, and because they are quite sensitive, they should be useful for measuring dipolar couplings in other RNA molecules. Two recent publications (Zidek et al., 2001; Yan et al., 2002) presented pulse sequences for the measurement of other dipolar couplings in nucleic acid sugars and bases. Use of their pulse sequences in combination with the ones described above will increase the number of measured couplings and improve the precision of RNA structures determined by NMR.

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