Journal of Biomolecular NMR, **19:** 367–370, 2001.
KLUWER/ESCOM

Measurement of $^{1}H3'$-^{31}P dipolar couplings in a DNA oligonucleotide by constant-time NOESY difference spectroscopy

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Received 30 January 2001; Accepted 14 February 2001

Key words: constant-time NOESY, Dickerson dodecamer, dipolar coupling, liquid crystal, Pf1

Abstract

The ratios of cross peak intensities in a selective constant-time NOESY experiment, recorded with and without ^{31}P decoupling, yield values for the sum of the H3′-P scalar and dipolar couplings. The selective refocusing of H3′ resonances in this experiment results in excellent resolution and sensitivity, even in the liquid crystalline phase where the ^{1}H spectrum is broadened by unresolved homonuclear dipolar couplings. The vicinal H3′-P scalar and dipolar couplings in the DNA oligomer d$(CGCGAATTCGCG)_2$ were measured in both isotropic solution, and in a liquid crystalline phase. Isotropic values are in good agreement with values reported previously. Dipolar couplings are in excellent agreement with the NMR structure for this dodecamer, and to a somewhat lesser extent with the X-ray structures.

Although DNA oligomers were among the first biological macromolecules for which a structure was reported by NMR (Clore and Gronenborn, 1985; Hare and Reid, 1986), the paucity of protons and the near-absence of experimental long-range constraints in such systems make accurate structure determination notoriously difficult (Schmitz and James, 1995; Allain and Varani, 1997). One-bond dipolar couplings can alleviate this problem by providing global constraints on the orientations of bond vectors relative to a single alignment frame (Kung et al., 1995; Tolman et al., 1995; Tjandra et al., 1997; Bayer et al., 1999). Measurement of such couplings has been greatly facilitated by the introduction of dilute liquid crystalline media that allow tuning of the molecular alignment to a range where measurement of the dipolar couplings is easiest (Tjandra and Bax, 1997; Clore et al., 1998; Hansen et al., 1998). Even with the multitude of such one-bond dipolar couplings now accessible, the structure remains experimentally underdetermined because one-bond dipolar interactions do not provide translational information, and NOE distance information is generally insufficiently accurate to prevent the accumulation of small errors. Recent experiments that can detect internucleotide H-bonding interactions or dipolar couplings solve part of this problem by tightly constraining the distance between H-bonded nucleotides (Dingley and Grzesiek, 1998; Pervushin et al., 1998; Wu et al., 2001). Nevertheless, none of these experiments provide direct information on the conformation of the phosphodiester linkage between nucleotides. Experimental information is usually limited to the $C4'_i$-$C3'_i$-$O3'_i$-P_{i+1} torsion angle, ε, for which a Karplus parameterization is available (Lankhorst et al., 1984). More recently, a method was introduced which derives information on the torsion angles α and ζ from C-H dipolar, ^{31}P-CSA relaxation interference (Richter et al., 2000).

Here, we show that it is readily possible to measure the $^{1}H3'$-^{31}P dipolar coupling when the oligonucleotide is dissolved in a liquid crystalline medium. The favorable gyromagnetic ratio of ^{31}P partially compensates for the typically rather long $^{1}H3'$-^{31}P distance, resulting in couplings that are readily measurable. A range of different methods for measurement of $^{3}J_{H3'\text{-}P}$ couplings has been proposed in the literature.

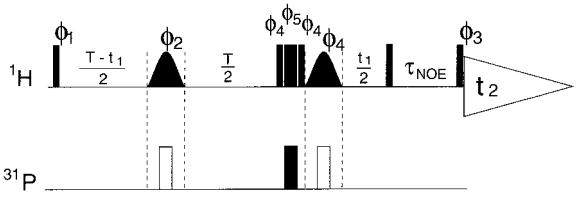

Figure 1. Pulse sequence of the CT-NOESY ^{31}P difference experiment for measurement of $^3J_{H3'P}$ and $D_{H3'P}$ couplings in oligonucleotides. The experiment is executed twice, in an interleaved manner, once without the open ^{31}P 180° pulses (reference experiment), and once with all three ^{31}P 180° pulses included (attenuated experiment). The $90^\circ_{\phi4}$-$180^\circ_{\phi5}$-$90^\circ_{\phi4}$ ^{1}H pulse is of the composite type, and is also phase cycled. Unless marked, pulses are applied with phase x. Phase cycling: ϕ_1 = x,−x; ϕ_2 = x,x,y,y,−x,−x,−y,−y; ϕ_3 = 4(x),4(y),4(−x),4(−y); ϕ_4 = 16(x),16(y),16(−x),16(−y); ϕ_5 = 16(y),16(−x),16(−y), 16(x); Receiver = x,2(−x),x,y,2(−y),y,−x,2(x),−x,−y,2(y),−y. The extensive phase cycle was used because the inverse probe employed in this study was not equipped with pulsed field gradients. The shaped ^{1}H 180° pulses are of the RE-BURP type (Geen and Freeman, 1991), and ^{31}P pulses are applied at their midpoint. Dephasing efficiency during the RE-BURP pulse is measured to be 90% relative to dephasing during free precession. The RE-BURP pulse duration was 10 ms, for an effective (>90%) inversion bandwidth of ±200 Hz, and negligible inversion at offsets larger than 325 Hz from the carrier.

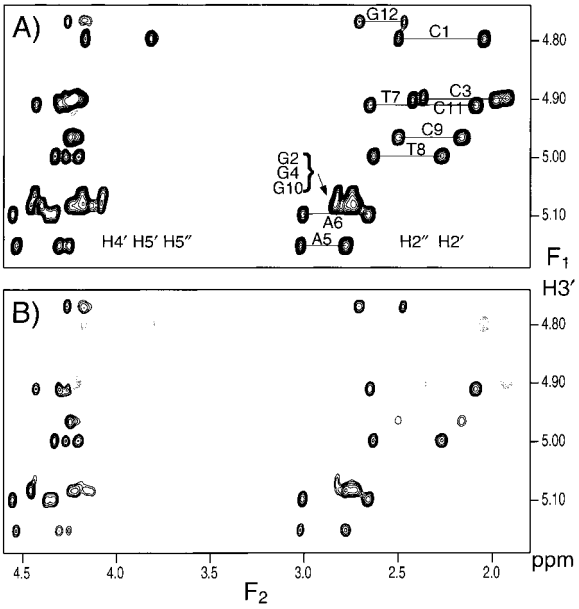

Figure 2. Upfield region of the 600 MHz CT-NOESY spectra of d(CGCGAATTCGCG)$_2$ recorded with the pulse scheme of Figure 1 (A) in the presence and (B) in the absence of ^{31}P decoupling during the constant time evolution period. The T duration was 70.01 ms, and τ_{NOE} = 300 ms. The acquired data matrix consisted of two interleaved sets of 71* × 1024* each, recorded with 96 scans per FID, and a combined total measuring time of 12.5 h. Spectra were separated and processed with NMRPipe (Delaglio et al., 1995), apodized with a 72°-shifted sine bell in t_1, and a 72°-shifted squared sine bell in t_2, and zero-filled to yield a 1024×2048 matrix for the final part of the real spectrum. Dashed contours correspond to negative intensity.

These include experiments that measure splittings directly from spectra that are enhanced by the use of band-selective pulses, employing either ^{31}P or ^{1}H detection (Sklenar and Bax, 1987). Another approach obtains the coupling from constrained fitting of the ^{13}C3′-^{1}H3′ multiplet recorded with different modes of ^{31}P decoupling (Schwalbe et al., 1994). A third class of experiments utilizes quantitative J-correlation (Bax et al., 1994), and derives the coupling from the difference in cross peak intensity in the presence and absence of ^{31}P decoupling, either in a homonuclear CT-COSY experiment (Clore et al., 1998) or in heteronuclear ^{13}C-^{1}H CT-HMQC schemes (Hu et al., 1999; Szyperski et al., 1999). The method described here is a NOESY variant of the CT-COSY experiment. As will be shown below, it offers improved resolution and allows ^{1}H-^{31}P couplings to be measured from multiple cross peaks, resulting in a more complete set of measured couplings.

The pulse scheme is shown in Figure 1. The $180^\circ_{\phi2}$ pulse is of the RE-BURP type (10 ms at 600 MHz) and covers the 0.5 ppm region of the H3′ protons, leaving most H4′ and all other protons unaffected. So, homonuclear couplings to H3′ are refocused at time t_1 prior to the end of the constant-time evolution period, of total duration T. At the midpoint of the final t_1 fraction of this T period, couplings to H3′ are removed by application of a non-selective composite $90^\circ_{\phi4}$-$180^\circ_{\phi5}$-$90^\circ_{\phi4}$ inversion pulse, followed by a H3′-selective RE-BURP pulse. This combination results in no net effect on H3′, but inverts all other spins, thereby effectively decoupling H3′ from all other protons during the latter t_1 fraction of the T period. The net result is that, in the absence of the open ^{31}P 180° pulses, all J and dipolar couplings to H3′ are refocused at the end of the constant time evolution period, whereas chemical shift evolution has occurred for a time t_1. In the presence of the open 180° ^{31}P pulses, ^{1}H3′-^{31}P J and dipolar couplings evolve for a duration $T_{eff} = T + 2 \times \tau_{180} \times 0.9$, where τ_{180} is the duration of the H3′ RE-BURP pulse. The scaling factor 0.9 is determined experimentally and accounts for ^{1}H3′-^{31}P coupling evolution during the RE-BURP pulse, when the ^{31}P 180° pulse is applied at the center of the RE-BURP. The duration of the NOESY mixing time, τ_{NOE}, is chosen quite long, such that cross peak intensities are near or just past their maximum. Spin

diffusion to base and H1′ protons can actually be helpful in the analysis when cross peaks to H2′, H2″and H4′ protons yield too much overlap.

Figure 2 compares the H3′ → H4′/H5′/H5″ and H2″/H2′ regions, recorded with and without ^{31}P decoupling, for a sample containing 0.6 mM d(CGCGAATTCGCG)$_2$, pH 6.8 (uncorrected meter reading), 100 mM NaCl, in 99.5% D_2O. The sample also contained 20 mg Pf1/ml (ASLA http://130.237.129.141//asla/asla-phage.htm), causing the H3′ resonances to be quite broad as a result of the multiple unresolved homonuclear dipolar couplings and leading to severe overlap in the regular 2D NOESY at 600 MHz (data not shown). In contrast, as can be seen in Figure 2, resolution in the F_1 dimension is excellent and only limited by the length of the constant-time evolution period. Although resolution in this truncated constant-time dimension can be enhanced further by means of linear prediction, we chose not to do this because linear prediction tends to increase uncertainty in the peak amplitudes, which contains the information needed for deriving $^3J_{H3'P} + D_{H3'P}$.

The ratio of the amplitude in the attenuated spectrum, with all three ^{31}P pulses included, relative to the reference spectrum equals $\cos[\pi(^3J_{H3'P} + D_{H3'P})T_{eff}]$. The signal-to-noise ratio obtainable per unit of total measuring time in the CT-NOESY spectrum is proportional to $\exp(-R_2T)$, where R_2 is the transverse relaxation rate of H3′ and T the constant-time duration. So, in the limit where $|^3J_{H3'P} + D_{H3'P}| \ll R_2$, the intrinsic accuracy of the $^3J_{H3'P} + D_{H3'P}$ derived from the intensity ratios is highest when $T_{eff} \approx 2/R_2$. In the case of the dodecamer studied here, transverse relaxation is rather slow ($R_2 \approx 20\ s^{-1}$), and a slightly shorter value for T_{eff} (88 ms) was used.

As can be seen in Figure 2, for C1 and C3 the value of $\pi(^3J_{H3'P} + D_{H3'P})T_{eff}$ is larger than $\pi/2$, causing the intensities for all cross peaks to these H3′ resonances to be negative. For others (G2, G10, C11), the value is close to $\pi/2$, causing the intensity in Figure 2B to be near zero. Each pair of corresponding cross peaks in Figure 2 provides an independent measure for the value of $|^3J_{H3'P} + D_{H3'P}|$. The uncertainty in this coupling equals $[1 + \cos^2(\pi|^3J_{H3'P} + D_{H3'P}|T_{eff})]^{1/2} \times [\pi T_{eff} \sin(\pi|^3J_{H3'P} + D_{H3'P}|T_{eff}) \times S/N]^{-1}$, where S/N is the signal-to-noise ratio of the peak in the reference spectrum (Figure 2A). A weighted average of the ratios of all resolved cross peaks to a given H3′ provides optimum sensitivity for the measurement of the coupling. Values for $^3J_{H3'P}$ and $D_{H3'P}$ with their associated uncertainties are listed in Table 1. The $^3J_{H3'P}$ are in excellent agreement (rmsd = 0.25 Hz) with those from a less complete set reported previously (Sklenar and Bax, 1987).

Table 1. Measured scalar and dipolar ^{1}H3′-^{31}P couplings in d(CGCGAATTCGCG)$_2$

Residue	$J_{H3'P}$ (Hz)	$D_{H3'P}$ (Hz)
C1	5.96±0.04[a]	1.28±0.08
G2	3.76±0.03	1.49±0.08
C3	5.30±0.04	1.94±0.11
G4	4.01±0.03	1.59±0.09
A5	3.01±0.06	1.58±0.11
A6	2.43±0.08	1.14±0.13
T7	2.74±0.08	0.89±0.14
T8	3.29±0.06	0.31±0.13
C9	5.16±0.04	−0.33±0.12
G10	3.96±0.03	−0.28±0.09
C11	5.32±0.04	0.25±0.09

[a]Errors only reflect the uncertainty in the derived couplings from random noise in the peak intensities.

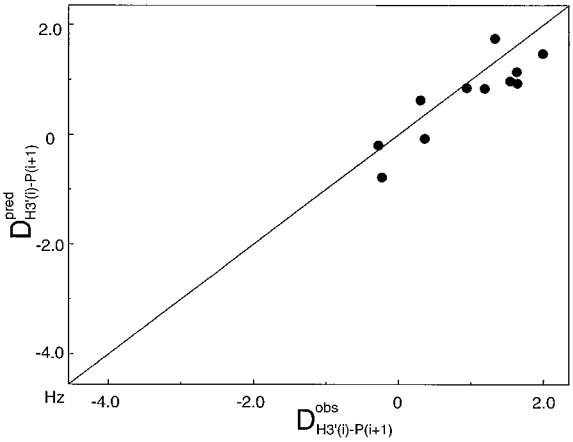

Figure 3. Comparison of $D_{H3'P}$ couplings measured in Pf1 with values predicted from the basis of the NMR structure (PDB entry 1DUF) (Tjandra et al., 2000). The experimental couplings span a narrow range relative to the possible allowed range of −4.2 to +2.2 Hz for an alignment tensor with $D_a^{CH} = 20$ Hz, R = 0.05, assuming $r_{H3'-P} = 2.85 \pm 0.15$ Å.

$D_{H3'P}$ values in d(CGCGAATTCGCG)$_2$, measured in Pf1, span a relatively narrow range, from −0.3 to +2.0 Hz. The alignment tensor for the dodecamer in Pf1 has the same orientation as in bicelles (Tjandra et al., 2000), but smaller rhombicity (0.05), as determined from measurement of one-bond ^{13}C-^{1}H dipolar couplings. The magnitude of the alignment tensor was

measured to be $D_a^{CH} = 20$ Hz. The H3′-P distance is relatively uniform in B-DNA (2.85±0.15 Å), and $D_{H3'P}$ couplings for this degree of alignment are expected to fall in the −4.3 to +2.2 range in Pf1. The +2 Hz value corresponds to an H3′-P orientation orthogonal to the DNA axis, and −4 Hz to a parallel orientation. Figure 3 compares measured $D_{H3'P}$ values with those predicted for the NMR structure (Tjandra et al., 2000). With a Pearson's correlation coefficient R = 0.87 and a pairwise rmsd of 0.4 Hz, the agreement is somewhat better than for either the original X-ray structure of this dodecamer (Dickerson and Drew, 1981) (PDB entry 1BNA; R = 0.63, rmsd 0.7 Hz) or the more recent high resolution structure (entry 355D; R = 0.66, rmsd 0.7 Hz) (Shui et al., 1998). The poorer correlation for the X-ray structures is likely resulting from small backbone distortions caused by intermolecular contacts and coordination of a Mg^{2+} ion in the crystal.

Clearly, $D_{H3'P}$ values are ideally suited for improving the accuracy of the phosphodiester linkage conformation in oligonucleotides. Despite their small absolute values, they can readily be measured at high accuracy. Work on refining the NMR structure of the dodecamer by inclusion of the $D_{H3'P}$ couplings, in addition to 170 quantitative ^{1}H-^{1}H dipolar couplings per strand and other novel experimental restraints, is in progress.

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