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Determination of Three-Bond $^{1}H3'–^{31}P$ Couplings in Nucleic Acids and Protein–Nucleic Acid Complexes by Quantitative *J* Correlation Spectroscopy

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A new sensitive two-dimensional quantitative *J* correlation experiment is described for measuring $^{3}J_{H3'-P}$ couplings in nucleic acids and protein–nucleic acid complexes. The method is based on measuring the change in intensity of the $^{1}H–^{1}H$ cross peaks in a constant-time $^{1}H–^{1}H$ COSY experiment which occurs in the presence and absence of $^{3}J_{H3'-P}$ dephasing during the constant-time evolution period. For protein–nucleic acid complexes where the protein is ^{13}C-labeled but the nucleic acid is not, ^{12}C-filtering is readily achieved by the application of a series of ^{13}C purge pulses during the constant time evolution period without any loss of signal-to-noise of the nucleic acid cross peaks. The method is demonstrated for the Dickerson DNA dodecamer and a 19 kDa complex of the transcription factor SRY with a 14mer DNA duplex. The same approach should be equally applicable to numerous other problems, including the measurement of $J_{H–Cd}$ couplings in cadmium-ligated proteins, or $^{3}J_{CH}$ couplings in other selectively enriched compounds.

Key Words: **$^{1}H–^{31}P$ couplings; quantitative *J* correlation; nucleic acids; protein–nucleic acid complexes.**

The three-bond $^{1}H3'–^{31}P$ *J* coupling in nucleic acids contains potentially important structural information on the ϵ (C4′–C3′–O3′–P) backbone torsion angle. The Karplus equation which relates ϵ to $^{3}J_{H3'-P}$ has been parametrized (*1–3*) and is given by $^{3}J_{H3'-P} = 15.3\cos^{2}(\epsilon + 120°) - 6.2\cos(\epsilon + 120°) + 1.5$ Hz (*1*). Its application for conformational analysis, however, has remained limited because of difficulties involved in accurate measurement of $^{3}J_{H3'-P}$ in larger nucleic acid fragments or in protein–nucleic acid complexes. In smaller systems, measurements have been made using either selective proton-flip experiments (a heteronuclear, 2D *J*-resolved experiment with either ^{1}H or ^{31}P detection), or ^{1}H-detected heteronuclear correlation spectroscopy (*4*). More recently a third approach, referred to as P-FIDS-CT-HSQC, has been introduced for ^{13}C-enriched oligonucleotides in which $^{3}J_{H3'-P}$ is obtained from a constrained fitting analysis of the $^{13}C3'–^{1}H3'$ cross peak shape (*5, 6*). The applicability of the earlier experiments (*4*) is limited by the extensive resonance overlap typically encountered in larger systems. The P-FIDS-CT-HSQC is restricted to ^{13}C-enriched nucleic acids and requires specialized software for extracting the *J* coupling in a reliable manner (*5, 6*).

In this paper, we present a simple and relatively sensitive 2D quantitative *J* correlation (*7*) experiment for determining H3′–^{31}P coupling constants. Its accuracy is tested by comparing $^{3}J_{H3'-P}$ values measured for the Dickerson DNA dodecamer with those measured previously using 2D *J*-resolved and heteronuclear correlation spectroscopy (*4*). Its applicability to larger molecular weight systems is demonstrated for a ~19 kDa complex of the transcription factor SRY with a 14mer duplex DNA.

The pulse sequence for determining the $^{3}J_{H3'-P}$ coupling by 2D quantitative *J* correlation spectroscopy is illustrated in Fig. 1. It is essentially a constant-time (CT) $^{1}H–^{1}H$ COSY experiment, executed with and without ^{31}P decoupling during the constant-time evolution period, of total duration $2T$ (ca. 50 ms; see later discussion). The two experiments are carried out in an interleaved manner, once with the position of the ^{31}P 180° pulse at time point *a*, and once at time point *b*. With the ^{31}P pulse at time point *a*, the $^{3}J_{H3'-P}$ coupling is refocused at the end of the $2T$ period. With the ^{31}P pulse at time point *b*, however, the $^{3}J_{H3'-P}$ coupling is active during the entire $2T$ period, so that the magnetization of H3′ protons is attenuated by a factor of $\cos(2\pi^{3}J_{H3'-P}T)$. Consequently, the value of $^{3}J_{H3'-P}$ is given by $[\cos^{-1}(I_{att}/I_{ref})]/2\pi T$, where I_{att} and I_{ref} are the intensities of the H3′(F_1)–H2′(F_2) or H3′(F_1)–H2″(F_2) cross peaks in the attenuated (position *b*) and reference (position *a*) spectra, respectively. For the case of a protein–nucleic acid complex, in which the protein is uniformly labeled with ^{13}C but the nucleic acid is not, a series of three 90° ^{13}C purge pulses (*8, 9*) applied during the CT evolution period can be used to eliminate all signals from protons attached to ^{13}C, without any loss in signal-to-noise for the nucleic acid cross peaks.

The signal intensity observed in the difference spectrum is a function of the duration of the constant-time period, $2T$. For the H3′(F_1)–H2′(F_2) cross peak in the reference spectrum, the

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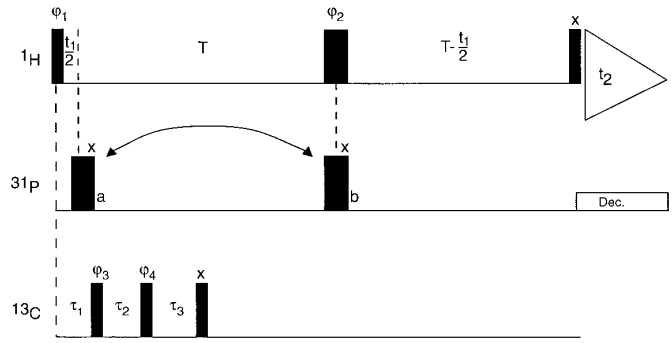

FIG. 1. Pulse scheme for the ^{12}C-filtered CT ^{1}H–^{1}H{^{31}P} COSY difference experiment. Narrow and wide pulses denote 90° and 180° flip angles, respectively. The reference spectrum is recorded with the ^{31}P 180° pulse labeled *a* and omitting the pulse labeled *b*, whereas the attenuated spectrum is recorded using pulse *b* and omitting pulse *a*. Unless indicated otherwise, all pulses are applied along the *x*-axis. The phase cycle is as follows: $\phi_1 = x, -x$; $\phi_2 = 2(x), 2(y), 2(-x), 2(-y)$; $\phi_3 = 2(x), 2(-x)$; $\phi_4 = 4(x), 4(-x)$; receiver = $x, 2(-x), x$. Quadrature detection in t_1 is obtained by the States-TPPI technique, incrementing ϕ_1. The delays τ_1, τ_2, τ_3, and T are set to 3, 3.5, 4, and 25 ms, respectively.

signal is proportional to $\sin(2\pi^3 J_{H3'H2'}T)\cos(2\pi^3 J_{H3'H2''}T)\cos(2\pi^3 J_{H3'H4'}T)\exp(-2T/T_2)$, where T_2 is the transverse relaxation time of H3′. The attenuated spectrum is weaker by a factor $\cos(2\pi^3 J_{H3'-P}T)$. Thus, the signal-to-noise in the difference spectrum is proportional to $\sin(2\pi^3 J_{H3'H2'}T)\cos(2\pi^3 J_{H3'H2''}T)\cos(2\pi^3 J_{H3'H4'}T)[1 - \cos(2\pi^3 J_{H3'-P}T)]\exp(-2T/T_2)$. In the limit of large systems with very short H3′ T_2 values, where $\pi J T_2 \ll 1$, with $J = {}^3J_{H3'H2'}$, ${}^3J_{H3'H2''}$, ${}^3J_{H3'H4'}$, ${}^3J_{H3'-P}$, this function is at a maximum for $2T = 3T_2$. For smaller systems, the trigonometric terms need to be taken into account explicitly, and a relatively broad maximum near $2T \approx 50$ ms is obtained. Note that in B-form DNA, which has a C2′-endo sugar pucker, ${}^3J_{H3'H2''}$ typically is much smaller than ${}^3J_{H3'H2'}$, and the H3′–H2″ cross peak is much weaker than the H3′–H2′ peak. The reverse applies to sugars with C3′-endo sugar pucker, which show more intense H3′–H2″ cross peaks. In practice, couplings between H5′ or H5″ and ^{31}P are usually not easily measured using the present experiment, as cross peaks involving these resonances are typically poorly resolved in the COSY spectrum.

The method is demonstrated for a 5 mM sample of the Dickerson dodecamer 5′d(CGCGAATTCGCG)$_2$ and a 1 mM sample of the ~19 kDa SRY–DNA complex recorded in D_2O on a Bruker DMX500 spectrometer. The sequence of the 14mer duplex DNA used in the SRY–DNA complex is 5′d(CCTGCACAAACACC)5′d(GGTGTTTGTGCAGG) and the SRY protein is uniformly (>98%) ^{13}C-labeled. Figure 2 illustrates the H3′(F_1)–H2′/H2″(F_2) region of the reference and difference (reference minus attenuated) CT ^{1}H–^{1}H{^{31}P} COSY spectra obtained for the Dickerson dodecamer (A and B, respectively) and the SRY–DNA complex (C and D, re-

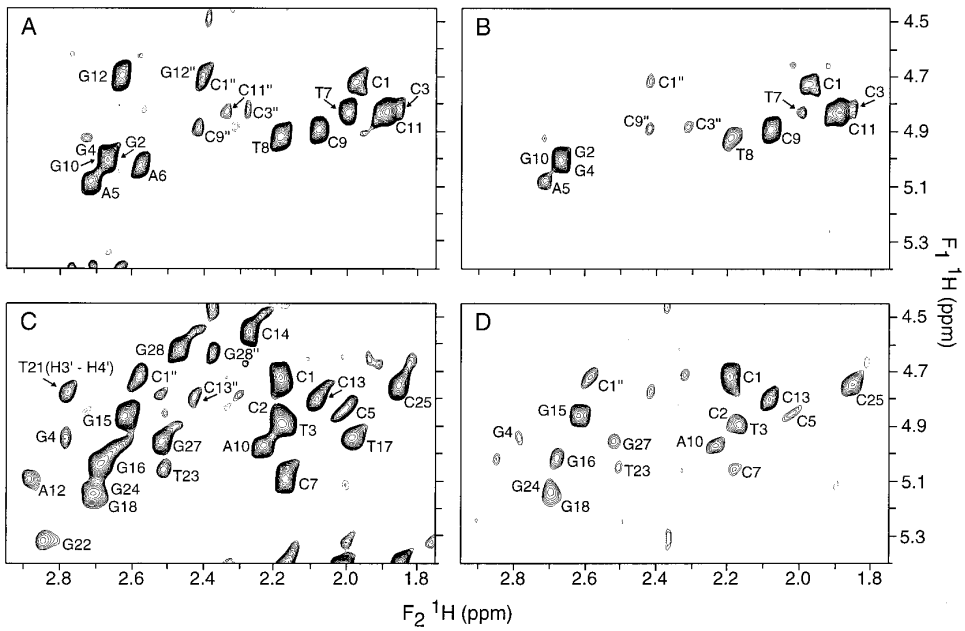

FIG. 2. H3′(F_1)–H2′/H2″(F_2) region of the reference and difference (reference minus attenuated) CT ^{1}H–^{1}H{^{31}P} COSY spectrum recorded at 500 MHz on the Dickerson DNA dodecamer (A and B, respectively) and the SRY–DNA complex (C and D, respectively). The reference and attenuated spectra were recorded in an interleaved manner with acquisition times of 50 ms(t_1) and 63.3 ms (t_2), and spectral widths of 6009 Hz (F_1) and 8090 Hz (F_2). The starting contour level in B is a factor of 2 lower than in A. C and D are plotted at the same contour levels. H3′(F_1)–H2′(F_2) peaks are labeled according to nucleotide number; H3′(F_1)–H2″(F_2) peaks are indicated by a double prime following the nucleotide number. Note the presence of the T21(H3′–H4′) cross peak in C with an unusually upfield-shifted H4′ resonance as a result of a large ring current effect arising from the close proximity of Phe12 in SRY (*17*). The total measuring time was 14.5 h for the Dickerson DNA dodecamer (5 mM) and 43.5 h for the SRY–DNA complex (1 mM).

spectively). Since the t_1 evolution period is of the constant-time type, the proton multiplets are collapsed into singlets in the F_1 dimension (*10*). In the F_2 dimension, the regular ^{1}H–^{1}H multiplet shape is present, in which the ^{1}H2′ (or ^{1}H2″) signal of interest, which gives rise to the H3′(F_1)–H2′(F_2) [or H3′(F_1)–H2″(F_2)] cross peak, is antiphase with respect to H3′, but in-phase relative to all other protons. The spectrum is most conveniently phased to be dispersive in the F_2 dimension, such that the unresolved antiphase H2′–{H3′} multiplet has the appearance of a strongly resolution enhanced absorptive resonance in this dimension (*11, 12*).

$^3J_{H3'-P}$ couplings derived from Figs. 2A and 2B for the Dickerson dodecamer are listed in Table 1. These values have been calculated simply using peak heights in the reference and attenuated spectra, and the error corresponds to the uncertainty induced by the presence of noise, the rms value of which is measured in an empty region of the spectrum. The agreement between the present results and previously reported (*4*) values is excellent, with a pairwise root-mean-square difference of only 0.4 Hz. As is invariably the case when J couplings are measured by techniques other than those based on the E.COSY principle (*13, 14*), the uncertainty is largest for the smallest couplings. Thus, as expected, the difference between the present and previously reported values is largest for A6, the nucleotide with the smallest $^3J_{H3'-P}$ coupling.

For the 14mer complexed with SRY, the observed couplings range from ≤1 to 7 Hz (Table 1), consistent with ϵ torsion angle values of 167° to 205°, respectively. Clearly, because of the multi-valued character of the Karplus curve, ϵ values in the 275° to 313° range are also compatible with the measured $^3J_{H3'-P}$ values, but such ϵ values are rare in deoxyribonucleotides. Measurement of $^3J_{CP}$ values is needed to unambiguously rule out this latter range of couplings (*5, 15, 16*). In principle, ϵ values near 130° and −10° could also give rise to J couplings in the range observed in our study, but these are sterically strongly forbidden.

In conclusion, we have shown that $^3J_{H3'-P}$ couplings can be obtained in a reliable and straightforward manner for nucleic acids and protein–nucleic acid complexes. The method is of reasonable sensitivity, even for larger molecular weight (~20 kDa) systems, because transverse relaxation of the H3′ proton is relatively slow, considering that the H3′ proton typically has a strong dipolar interaction to only a single proton (H2′) to which it has a short (<2.5 Å) distance. Under favorable circumstances, we therefore anticipate that the CT ^{1}H–^{1}H{^{31}P} COSY difference experiment should be applicable to complexes considerably larger than the 19 kDa SRY–DNA complex. The method is not restricted to measuring $^3J_{HP}$ couplings in nucleic acids, but should be equally applicable to numerous other problems, including the measurement of J_{H-Cd} couplings in cadmium-ligated proteins, or $^3J_{CH}$ couplings in other selectively enriched compounds.

TABLE 1

$^3J_{H3'-P}$ Couplings Obtained for the Dickerson DNA Dodecamer and for the SRY–DNA Complex Using the CT ^{1}H–^{1}H{^{31}P} COSY Difference Experiment

C3′–H nucleotide	CT ^{1}H–^{1}H{^{31}P} COSY	Average value from other methods[a]
A. Dickerson DNA dodecamer		
C1	5.7 ± 0.3	6.4 ± 0.06
G2	*b*	3.6 ± 0.2
C3	5.1 ± 1.2[c]	5.7 ± 0.2
G4	*b*	4.0 ± 0
A5	3.2 ± 0.3	2.9 ± 0.4
A6	1.6 ± 0.9	2.6 ± 0.4
T7	2.3 ± 0.3	2.7 ± 0.4
T8	3.1 ± 0.2	3.0 ± 0.4
C9	5.0 ± 0.1	5.0 ± 0.06
G10	*b*	4.1 ± 0.2
C11	4.9 ± 1.0[c]	5.2 ± 0.06
B. 14mer complexed to SRY[d]		
C1/—	5.0 ± 0.2/ —	
C2/G27	*e* /4.5 ± 0.7	
T3/A26	*e* / —	
G4/C25	7.0 ± 1.4/5.6 ± 0.4	
C5/G24	4.8 ± 0.9/ *f*	
A6/T23	— /6.6 ± 0.6	
C7/G22	1.4 ± 0.9/ <1	
A10/T19	4.9 ± 0.6/ —	
C11/G18	— / *f*	
A12/T17	3.6 ± 2.0/3.8 ± 1.6	
C13/G16	5.6 ± 0.3/4.4 ± 0.4	
—/G15	— /5.1 ± 0.3	

[a] The other methods published by Sklenar and Bax (*4*) consist of proton-flip experiments using 2D J-resolved (^{1}H or ^{31}P detected) and ^{1}H-detected heteronuclear correlation schemes.

[b] The H3′–H2′ cross peaks of G2, G4, and G10 of the Dickerson DNA dodecamer are overlapped, and the value of $^3J_{H3'P}$ for these residues could not be measured unambiguously.

[c] Measured from the H3′(F_1)–H2″(F_2) cross peaks since the corresponding H3′(F_1)–H2′(F_2) cross peaks are overlapped.

[d] The value for the sense strand (nucleotides 1–14) is given first, and that for the antisense strand (nucleotides 15–28) second.

[e] The H3′–H2′ cross peaks of C2 and T3 of the SRY 14mer are overlapped, and values of $^3J_{H3'P}$ for these residues could not be determined.

[f] The H3′–H2′ cross peaks of G18 and G24 of the SRY 14mer are overlapped, and values of $^3J_{H3'P}$ for these residues could not be determined.

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