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Measurement of $^3J_{C2'P}$ Scalar Couplings in a 17 kDa Protein Complex with ^{13}C,^{15}N-Labeled DNA Distinguishes the B_I and B_{II} Phosphate Conformations of the DNA

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Since the phosphodiester backbone in DNA[1] duplexes shows a pronounced scarcity of $^1H-^1H$ NOEs,[2] measurements of vicinal scalar coupling constants have an important role for high-quality DNA structure determinations.[3,4] In this Communication we present data on $^3J_{C2'P}$ couplings that provide constraints for the torsion angles ϵ (Figure 1)[5] in a free DNA duplex and a protein−DNA complex and enable investigations of the B_I/B_{II} conformational polymorphism of the DNA. X-ray structures had previously revealed the prevalence of these two distinctly different phosphodiester conformations in B−DNA duplexes.[6] In B_I, ϵ is *trans* and the succeeding backbone dihedral angle ζ is *gauche*$^-$, while in B_{II} ϵ is *gauche*$^-$ and ζ is *trans*. The B_{II} conformation was found to occur only in rare instances and to correlate with a significant widening of the minor groove.[6]

Two 14-base pair DNA-duplexes containing the *BS2* operator sequence[7] were studied.[8] One duplex was fully ^{13}C,^{15}N-labeled, and a partially labeled duplex contained ^{13}C and ^{15}N only in those nucleotides which contact the protein in the *Antp*(C39S)−DNA complex.[9] The $^3J_{C2'P}$ values were measured in the free DNA and in the complex with *Antp*(C39S), using 2D {^{31}P}-*sedct*-[^{13}C,^1H]-HSQC.[10] This approach relies on measurements of the signal attenuation of cross peaks from 2′CH_2 that originates from the passive $^3J_{C2'P}$. Cross peaks with 1′CH are not affected by scalar couplings to ^{31}P and can therefore be used as an internal reference, and cross peaks with 4′CH of the chain-terminal nucleotides can serve as standards that represent large ^{31}P couplings (Figure 2). Since the attenuation from the

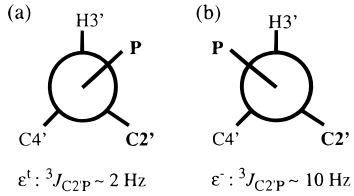

Figure 1. Newman projections of the torsion angle ϵ, where C2′ and the phosphorous atom are depicted in bold: (a) *trans* and (b) *gauche*$^-$. The $^3J_{C2'P}$ scalar couplings expected for these staggered rotamers[3] are given at the bottom. The *gauche*$^+$ conformation, which would also yield small $^3J_{C2'P}$ couplings, is energetically unfavorable.[11]

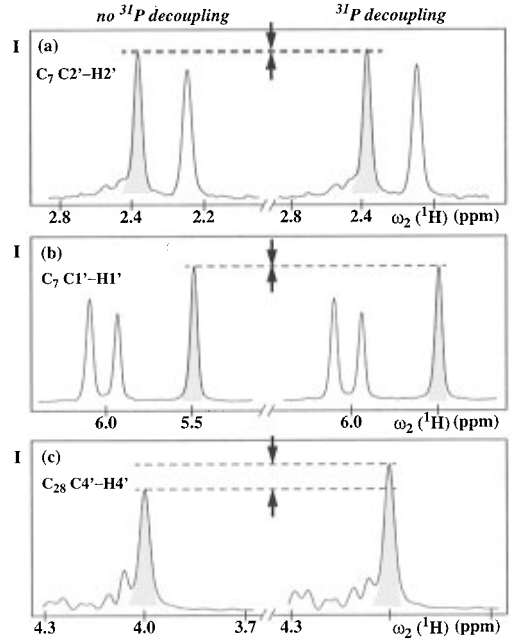

Figure 2. Cross sections along $\omega_2(^1H)$ through the cross peaks of Cyt 7 from the component spectra of 2D {^{31}P}-*sedct*-[^{13}C,^1H]-HSQC experiments[10] recorded with the fully ^{13}C,^{15}N-labeled DNA duplex in the *Antp* HD−DNA complex (concentration 1.5 mM, $T = 36$ °C, pH = 6.0) without (on the left) and with (on the right) ^{31}P decoupling. The peaks belonging to C_7 are highlighted in gray. The arrows indicate the difference in peak height with and without ^{31}P decoupling, which is the quantity of interest (see text). Part a shows cross sections containing the C2′−H2′ cross peak of C7, which is subject to a ^{31}P coupling of $^3J_{C2'P} = 2.5$ Hz. Part b shows the first internal reference: cross sections containing the C1′−H1′ cross peak of C7, which is not subject to a ^{31}P coupling. Part c shows the second internal reference: cross sections containing the C4′−H4′ cross peak of the 3′-terminal C_{28}, which is subject to a ^{31}P coupling of $^3J_{C4'P} = 8.9$ Hz. The chain terminal C_{28} was chosen for this internal reference because C4′ is subject to two ^{31}P couplings in all nonterminal nucleotides. The ^1H chemical shifts are relative to internal DSS. The spectra were recorded on a Varian Unity750+ spectrometer equipped with a ^1H-{^{13}C,^{31}P} triple resonance probehead, using a *ct* delay of 23 ms. A total of 240(t_1) × 512(t_2) complex points were accumulated, with $t_{1,max} = 22.5$ ms and $t_{2,max} = 57.0$ ms, yielding a measurement time of 22 h per spectrum. Prior to Fourier transformation the data matrix was extended by linear prediction to 340 complex points along t_1, and then multiplied with a cosine window in t_1 and a sine window shifted by 70° in t_2.[12]

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(1) Abbreviations used: NMR, nuclear magnetic resonance; 2D, two-dimensional; *sedct*, spin−echo difference constant time; HSQC, heteronuclear single quantum coherence; DNA, deoxyribonucleic acid; *Antp*, *Antennapedia*; HD, homeodomain; *BS2*, operator binding site 2; *Antp*(C39S), mutant 68-residue *Antp* homeodomain polypeptide with the homeodomain sequence in positions 1 to 60 and Cys 39 replaced by Ser; DSS, 2,2-dimethyl-2-silapentane-5-sulfonate.

(2) Wüthrich, K. *NMR of Proteins and Nucleic Acids*; Wiley: New York, 1986.

(3) Wijmenga, S. S.; Mooren, M. M. W.; Hilbers, C. W. In *NMR of Macromolecules*, (Roberts, G. C. K., Ed. Oxford University Press: Oxford, 1993; pp. 217−288.

(4) Tate, S.; Kubo, Y.; Ono, A.; Kainosho, M. *J. Am. Chem Soc.* **1995**, *117*, 7277−7278.

(5) Saenger, W. *Principles of Nucleic Acid Structure*; Springer: New York, 1984.

(6) (a) Cruse, W. B. T.; Salisbury, S. A.; Brown, T.; Cosstick, R.; Eckstein, F.; Kennard, O. *J. Mol. Biol.* **1986**, *192*, 891−905. (b) Dickerson, R. E. in *Structure & Methods*; (Sarma, R. H., Sarma, M. H., Eds.; Adenine Press: New York, 1990; Vol. 3, pp. 1−38. (c) Kennard, O.; Hunter, W. N. *Angew. Chem., Int. Ed. Engl.* **1991**, *30*, 1254−1277.

(7) Müller, M.; Affolter, M.; Leupin, W.; Otting, G.; Wüthrich, K.; Gehring, W. J. *EMBO J.* **1988**, *7*, 4299−4304.

(8) Fully and partially ^{13}C,^{15}N-labeled DNA oligomers were synthesized on a DNA synthesizer (Applied Biosystems Model 392-28) by the solid-phase phosphoramidite method, using isotopically labeled monomer units that had been synthesized according to the previously described general strategy [Ono, A; Tate, S; Kainosho, M. *Stable Isotope Applications in Biomolecular Structure and Mechanisms*; (Trewhella, J., Cross, T. A., Unkefer, C. J., Eds.; Los Alamos Natl. Lab.: New Mexico, 1994; pp. 127−144]. Approximately 1 mmol of oligomer was obtained from 5 mmol of nucleoside bound to the resin, and the purity of labeled oligomers was higher than 99% as estimated by HPLC analysis on a C-18 column (Inertsil ODS-2, GL Science). A full account of the synthesis will be presented elsewhere.

(9) Billeter, M.; Qian, Y. Q.; Otting, G.; Müller, M.; Gehring, W.; Wüthrich, K. *J. Mol. Biol.* **1993**, *234*, 1084−1097.

(10) Legault, P.; Jucker, F. M.; Pardi, A. *FEBS Lett.* **1995**, *362*, 156−160.

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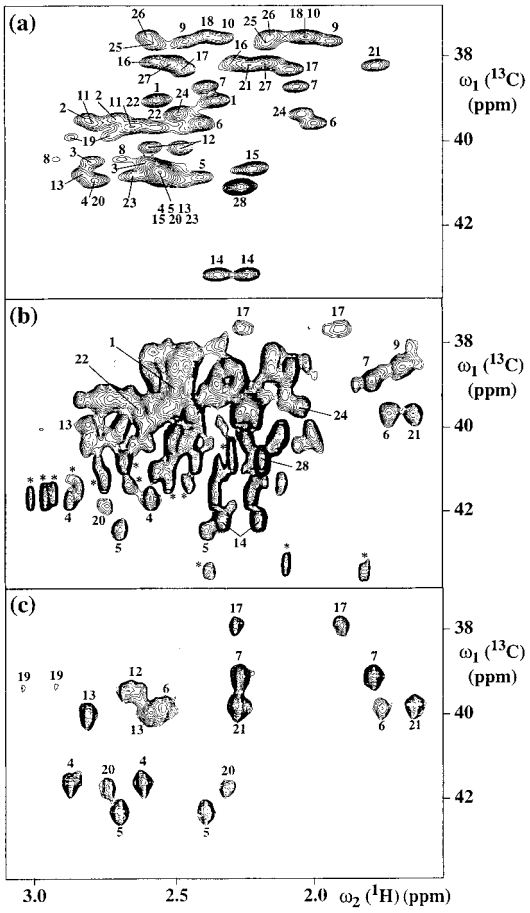

Figure 3. Spectral regions with C2′H resonances taken from the subspectrum without ^{31}P-decoupling of 2D *ct*-[^{13}C,^{1}H]-HSQC experiments: (a) fully ^{13}C,^{15}N-labeled free DNA duplex at a ^{1}H resonance frequency of 500 MHz; (b) fully ^{13}C,^{15}N-labeled DNA duplex in the *Antp*(C39S) HD−DNA complex at 750 MHz (peaks labeled with an asterisk are due to impurities); (c) partially ^{13}C,^{15}N-labeled DNA duplex in the Antp(C39S) HD−DNA complex at 750 MHz. ^{1}H and ^{13}C chemical shifts are in ppm relative to DSS. The spectrum a was recorded with a 1.5 mM solution of the DNA duplex (T = 36 °C, pH = 6.0, 50 mM KPO_4, 20 mM KCl) on a Bruker DRX 500 spectrometer equipped with a ^{1}H-{^{13}C,^{31}P} triple resonance probehead, using a *ct* delay of 46 ms. A total of 374 (t_1) × 512(t_2) complex points were accumulated with $t_{1,max}$ = 45.6 ms and $t_{2,max}$ = 85.6 ms, yielding a measurement time of 8.5 h per spectrum. Prior to Fourier transformation the data matrix was extended by linear prediction to 474 complex points along t_1, and then multiplied with a cosine window in t_1, and a sine window shifted by 70° in t_2.[12] For spectra b and c, which were recorded with a *ct* delay of 23 ms, the experimental procedures were as described in Figure 2 for the complex with the uniformly labeled DNA.

passive scalar coupling to ^{31}P is given by $\cos(\pi^3 J_{CP}\tau_{ct})$, intensity losses of 1.6% and 20%, respectively, correspond to $^3J_{C2'P}$ and $^3J_{C4'P}$ values of 2.5 and 8.9 Hz at a *ct* delay of 23 ms.

Compared to the use of 2D {^{31}P}-*sedct*-[^{13}C,^{1}H]-HSQC with RNA[10] the analysis for DNA benefits from the usually complete separation of the 2′CH_2 resonances from the remainder of the signals.[2] Since the ^{1}H2′ and ^{1}H2″ resonances are usually nondegenerate in DNA duplexes, each $^3J_{C2'P}$ coupling value can be measured twice (Figure 3). However, this also doubles the number of signals and may thereby introduce spectral overlap, which makes the use of partially labeled DNA attractive.

For the free DNA, 26 out of the total of 28 $^3J_{C2'P}$ couplings could be determined, and the resolution attained with a *ct* delay of 46 ms ensured that most couplings could be obtained with the fully ^{13}C,^{15}N-labeled DNA (Figure 3a). The $^3J_{C2'P}$ values were smaller than 2.7 Hz throughout (Figure 4). Since the *gauche*$^+$ conformation for ϵ is energetically unfavourable[11] this shows that all experimentally assessed deoxyribnucleotides adopt

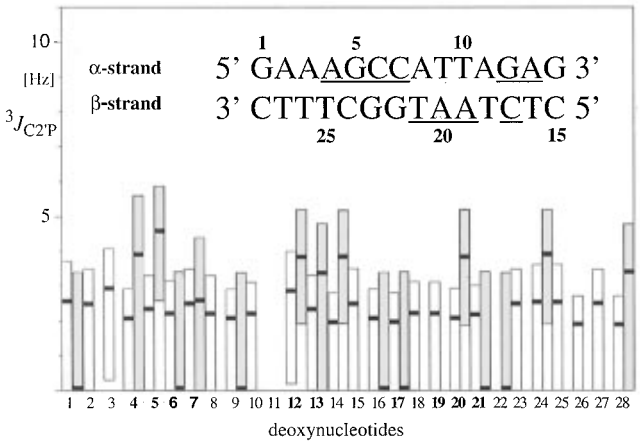

Figure 4. $^3J_{C2'P}$ values in the DNA 14mer duplex shown at the top. The deoxyribonucleotides that were labeled also in the partially ^{13}C,^{15}N-labeled DNA (see text) are underlined in the duplex structure and indicated by bold numbers at the bottom of the figure. The measured values are represented by black horizontal lines, and the experimental errors for the free DNA duplex are given by lightly shaded bars and for the *Antp*(C39S)HD−DNA complex by dark gray bars. Due to spectral overlap (Figure 3) a single averaged value is given for the pairs of deoxyribonucleotides 4 and 20, and 10 and 18, respectively. The experimental errors were derived from the standard deviation of the peak volumes of the C1′−H1′ cross peaks in two corresponding spectra.[10]

a *trans* conformation for ϵ (Figure 1), and thus a B_I conformation. In the *Antp*(C39S)−DNA complex, 16 out of the 28 $^3J_{C2'P}$ values could be determined, using a constant time delay of only 23 ms in 2D {^{31}P}-*sedct*-[^{13}C,^{1}H]-HSQC to adapt to the shorter T_2(^{13}C) in the complex. Here, the use of partially labeled DNA was mandatory to resolve overlap in the spectra (Figure 3b,c). All $^3J_{C2'P}$ values measured for the *Antp*(C39S)−DNA complex were smaller than 4.6 Hz (Figure 4), showing that the corresponding ϵ angles remain trans after complex formation and hence that protein−DNA interactions in this homeodomain system do not induce B_{II} conformations of the phosphodiester backbone.

In conclusion, 2D {^{31}P}-*sedct*-[^{13}C,^{1}H]-HSQC experiments[10] enabled the determination of $^3J_{C2'P}$ values in a DNA−protein complex of size 17 kDa. This allows derivation of constraints for the ϵ torsion angles,[2,3] and thus to contribute to the refinement of solution structures of DNA duplexes and their protein−DNA complexes. Due to an ambiguity in the corresponding Karplus relation, ϵ^t and ϵ^- (Figure 1) cannot be distinguished by analysis of $^3J_{H3'P}$, so that measurement of $^3J_{C2'P}$ is unique in providing a direct assessment of the intrinsic propensity of B−DNA duplexes in solution to adopt B_I or B_{II} conformations.[6] NMR structure determination may thus increasingly contribute to investigations on the role of modulation of the phosphodiester backbone conformation for protein−DNA recognition. For example, it will be of interest to investigate the significance of the all-B_I DNA conformation for protein−DNA recognition in further structure refinements of the *Antp* HD−DNA complex based on new data collection with the labeled DNA.

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(11) Pearlman, D. A.; Kim, S. H. *J. Biomol. Struct. Dyn.* **1986**, *4*, 49−67.

(12) DeMarco, A.; Wüthrich, K. *J. Magn. Reson.* **1976**, *24*, 201−204.