Measurement of Deoxyribose $^3J_{HH}$ Scalar Couplings Reveals Protein Binding-Induced Changes in the Sugar Puckers of the DNA

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$^3J_{HH}$ scalar couplings are most useful to assess the sugar puckers in NMR[1] structure determinations of DNA duplexes.[2,3] Such couplings can be obtained from 2D ^{1}H−^{1}H COSY,[3,4] but spectral overlap and mutual cancellation limit this approach to smaller systems. Above approximately 5 kDa, scalar couplings can be extracted from ^{13}C-resolved spectra of ^{13}C-labeled nucleic acids.[5−7] In this paper, we show that 3D ^{13}C-resolved in-phase [^{1}H,^{1}H]-COSY (3D HCH-COSY)[8] is a powerful experiment to derive values for $^3J_{1'2'}$, $^3J_{1'2''}$, $^3J_{2'3'}$,$^3J_{2''3'}$, and $^3J_{3'4'}$ in DNA, which yield constraints[10] for the deoxyribose dihedral angles[9] ν_1, ν_2, and ν_3. The couplings were determined for a uniformly ^{13}C/^{15}N-labeled 14-base pair DNA duplex prepared by solid-phase synthesis,[11] which binds to the *Antp(C39S)* homeodomain[12] to form a 17 kDa protein−DNA complex.[13]

3D HCH-COSY is a quantitative *J* correlation experiment[14] that yields $^3J_{HH}$ from intensity ratios of ^{13}C-resolved ^{1}H−^{1}H diagonal peaks and cross peaks.[8] Here, the experiment has been adapted for ^{13}C-labeled nucleic acids. The large spectral dispersion encountered for ^{13}C-labeled DNA favors the extraction of a large number of couplings. Measurements of $^3J_{1'2'}$ and $^3J_{1'2''}$ benefit from the large dispersion of C1′−H1′cross peaks, and measurements of $^3J_{2'3'}$,$^3J_{2''3'}$, and $^3J_{3'4'}$ benefit from the good separation of C3′−H3′ signals (Figure 1). This enables integration of most diagonal peaks {ω_1(C1′), ω_2(H1′), ω_3(H1′)} and {ω_1(C3′), ω_2(H3′), ω_3(H3′)}, which is essential for obtaining the couplings. These diagonal peaks are separated from the cross peaks with H2′ and H2″ chemical shifts along ω_2 (Figures 1 and 2). The separation of H3′ and H4′ resonances suffices to resolve most diagonal peaks {ω_1(C3′), ω_2(H3′), ω_3(H3′)} and the corresponding cross peaks that involve H4′ along ω_2, which enables the determination of $^3J_{3'4'}$ (Figures 1 and 3).

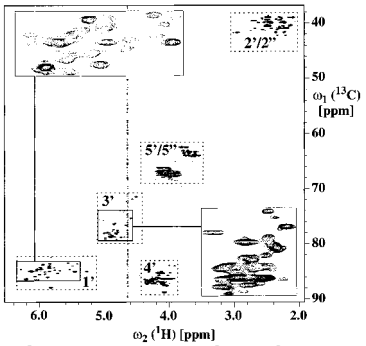

Figure 1. Spectral regions containing deoxyribose resonances from 2D *ct*-[^{13}C,^{1}H]-COSY[17] and 3D HCH-COSY spectra[8] of a uniformly ^{13}C,^{15}N-labeled DNA duplex in a complex with the *Antp*(C39S) homeodomain (concentration 1 mM, T = 36 °C, pH = 6.0). The dotted rectangles identify spectral regions of specified proton types. The two solid rectangles identify the regions of the 2D spectrum for which the corresponding spectral regions taken from a projection along ω_2(^{1}H) of the 3D spectrum are shown in the inserts. Chemical shifts are relative to internal DSS. The 3D HCH-COSY spectrum was recorded on a Bruker AMX500 spectrometer equipped with a ^{1}H-{^{13}C,^{31}P} triple-resonance probehead. The setup was as in ref 8, except that the parameters T^I = 4.631 ms and T^{II} = 7.853 ms were adapted to the deoxyribose carbon−carbon one-bond scalar couplings, thus yielding δ = 7.720 ms and δ'= 7.837 ms (see Figure 1 in ref 8). ^{31}P was decoupled with a WALTZ16 sequence[25] during the presence of transverse ^{13}C-magnetization; 84(t_1) × 55(t_2) × 256(t_3) complex points were accumulated in ∼3.5 days, with $t_{1,max}$(^{13}C) = 20.0 ms, $t_{2,max}$(^{1}H)= 13.6 ms, and $t_{3,max}$(^{1}H) = 63.9 ms. Prior to Fourier transformation, the data matrix was extended by linear prediction by 60 complex points along t_1, and 40 complex points along t_2. The 2D *ct*-[^{13}C,^{1}H]-COSY spectrum was recorded on a Varian Unity750+ spectrometer equipped with a ^{1}H-{^{13}C,^{15}N} triple-resonance probehead, using a constant-time delay of 23 ms; 245(t_1) × 512(t_2) complex points were accumulated in 2.4 h, with $t_{1,max}$(^{13}C) = 22.8 ms and $t_{2,max}$(^{1}H) = 55.5 ms.

For accurate determination of $^3J_{HH}$, one needs to consider that rapid ^{1}H−^{1}H spin flips lead to faster relaxation of antiphase magnetization when compared with in-phase magnetization.[8,15,16] We measured the relaxation times of the two-spin order H_zC_z ,T_{1zz}, from two 2D *ct*-[^{13}C,^{1}H]-COSY experiments[17] (recording times: 4 h for the free and 16 h for the complexed DNA) in which H_zC_z was stored for delays of either τ_1 = 1 ms or τ_2 = 31.1 ms after the first INEPT. Since T_1(^{13}C) ≫ T_{1zz}, the intensity ratio I_1 (τ_1)/I_2 (τ_2) provides an estimate for the apparent selective T_1 relaxation times of the protons.[16] For the free DNA, we obtained $T_{1,sel}$(H2′/H2″) = 160 ± 10 ms and $T_{1,sel}$(H4′) = 165 ± 10 ms for the nonterminal nucleotides. Hence, couplings derived via generation of magnetization that is antiphase with respect to H2′/H2″ or H4′

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(1) Abbreviations used: NMR, nuclear magnetic resonance; 2D, two-dimensional; 3D, three-dimensional; *ct*, constant-time; COSY, correlation spectroscopy; 3D HCH−COSY, 3D ^{13}C-resolved in-phase [^{1}H,^{1}H]-COSY; $^3J_{XY}$, vicinal scalar coupling between protons X and Y; DNA, deoxyribonucleic acid; *Antp*, *Antennapedia*; HD, homeodomain; *Antp(C39S)*, mutant 68-residue *Antp* homeodomain polypeptide with the homeodomain sequence in positions 1 to 60 and Cys39 replaced by Ser; DSS, 2,2-dimethyl-2-silapentane-5-sulfonic acid.

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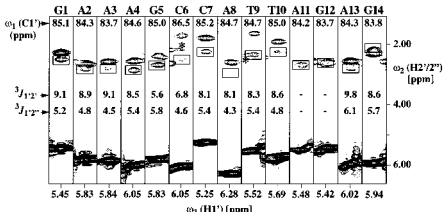

Figure 2. Contour plots of [ω_2(^{1}H), ω_3(^{1}H)] strips taken at the ^{13}C1′ chemical shifts (indicated at the top of the strips) from a 500 MHz 3D HCH-COSY spectrum[8] (see Figure 1) of the *Antp*(C39S) homeodomain-DNA complex. Along ω_3, the strips are centered about the H1′ chemical shifts. Except for G_{14}, the upper field and lower field cross peaks encode the H2′ and H2″ chemical shifts, respectively. The H2″ cross peaks are shown in boxes. The strips belong to the DNA strand with the nucleotide sequence indicated at the top. The diagonal peaks and the cross peaks have opposite sign.[8] Cross peaks belonging to spin systems of the other DNA strand are marked with an asterisk. Chemical shifts are relative to internal DSS. $^3J_{1'2'}$ and $^3J_{1'2''}$ values are given below the cross peaks (no data are given for A_{11} and G_{12} because the chemical shifts differences between H2′and H2″ are smaller than 0.1 ppm). To assess the experimental error, we performed the 3D HCH-COSY experiment twice. The average pairwise difference between the scalar couplings extracted from the two spectra was 0.4 Hz. Assuming a correction factor of 1.15 ± 0.05 to account for relaxation effects, we obtain, according to the Gaussian law of error propagation, an uncertainty between ±0.5 and ±0.7 Hz for couplings ranging from 2 to 9 Hz.

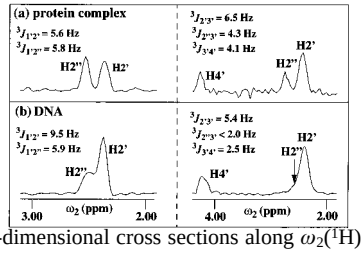

Figure 3. One-dimensional cross sections along ω_2(^{1}H) taken from 3D HCH-COSY spectra.[8] (a) Same 500 MHz spectrum of the *Antp*(C39S) homeodomain-DNA complex as shown in Figures 1 and 2. (b) Recorded for a 1 mM solution of the free DNA duplex (T = 36 °C, pH = 6.0, 50 mM KPO_4, 20 mM Cl). The cross sections on the left were taken at ω_1(^{13}C1′)/ω_3(^{1}H1′) of G_5, and the cross sections on the right at ω_1(^{13}C3′)/ω_3(^{1}H3′) of the same nucleotide. $^3J_{HH}$ values derived from these data are indicated. The change of the couplings upon protein binding is apparent from the change in relative cross peak intensities. For the free duplex, the spectrum was recorded on a Bruker DRX600 spectrometer equipped with a ^{1}H-{^{13}C, ^{15}N} triple-resonance probehead and processed as described in Figure 1, except that no ^{31}P-decoupling was applied; 87 (t_1) × 55(t_2) × 256(t_3) complex points were accumulated in 22 h, with $t_{1,max}$(^{13}C) = 20.7 ms, $t_{2,max}$(^{1}H) = 13.6 ms and $t_{3,max}$(^{1}H) = 63.9 ms.

are only about 3% smaller than the true couplings (see Figure 3 in ref 16). For the *Antp*(C39S) homeodomain−DNA complex, we obtained $T_{1,sel}$(H2′/H2″) = 100 ± 15 ms and $T_{1,sel}$(H4′) = 110 ± 20 ms, indicating a correction of about 8%.[18] For protons of a methylene group (H2′ and H2″) both coupled to a third proton (H1′ or H3′), spin flips between the methylene protons occurring during the dephasing period 2δ decrease the value measured for the larger coupling and increase the value for the smaller one.[19] This effect scales with the difference between the two couplings, which is generally smaller than about 5 Hz for deoxyribose rings.[3] Hence, such a correction is often not required. From ^{1}H−^{1}H NOESY spectra, the cross relaxation rates $\sigma_{2'2''}$ could be estimated to be about 4 s^{-1} for the free DNA and about 10 s^{-1} for the complex, respectively. We thus corrected only pairs of couplings in the complex which differed by more than 3 Hz (0.2 Hz was then subtracted from the smaller and added to the larger coupling).[20] Spin flips between H2′ and H2″ that occur during the rephasing period diminish the amount of magnetization refocused after a delay 2δ' by $\int_0^{2\delta'}\cos(\pi^3 Jt)\ e^{-\sigma_{2'2''}t}\ dt)/(\int_0^{2\delta'}\cos(\pi^3 Jt)\ dt$. Hence, except for $^3J_{3'4'}$, couplings ranging from 2 to 9 Hz have to be corrected further by 4−7% for the complex, and by 0−3% for the free DNA.

For the free DNA, 135 out of 136 $^3J_{HH}$ values (99%) involving nondegenerate H2′ and H2″ chemical shifts were determined (27 $^3J_{1'2'}$, 26 $^3J_{1'2''}$, 27 $^3J_{2'3'}$, 27 $^3J_{2''3'}$, and 28 $^3J_{3'4'}$). For the complex, 97 out of 128 $^3J_{HH}$ values (76%) were measured (22 $^3J_{1'2'}$, 21 $^3J_{1'2''}$, 16 $^3J_{2'3'}$, 16 $^3J_{2''3'}$, and 22 $^3J_{3'4'}$). The large number of couplings provides a sound basis for analysis of the deoxyribose conformations in free DNA and the protein−DNA complex.[3] This allows, for the first time, investigation of conformational changes of the sugar rings upon complexation with a protein in solution: Figure 3 provides evidence for a conformational shift induced at G_5, which exhibits numerous contacts with the protein.[13] Interpretation in the framework of a two-state model[21] assuming rapid interconversion of the C2′-*endo* and C3′-*endo* forms (pucker amplitude 35°) yields 85−95% and 45−70% C2′-*endo* conformer for the free DNA and the complex, respectively.[22]

The analysis of the sugar rings is of particular interest for B-type DNA. Although centered around C2′-*endo*, the deoxyribose conformations are broadly distributed from C3′-*exo* to O4′-*endo*, which contrasts with the much tighter clustering around C3′-*endo* in A-type helices.[21,23] Since this conformational polymorphism could play a role for sequence-specific protein−DNA recognition, use of the presently proposed approach for high-quality NMR structure determinations of DNA and their protein complexes is of interest. In X-ray structures, the determination of the sugar pucker is usually warranted only at a resolution of 2.0 Å or better.[21] Currently, there are only two X-ray structures of protein-DNA complexes in the Brookhaven Protein Databank for which the deoxyribose conformations were determined.[24] Hence, unique structural information about DNA conformation in protein complexes can be obtained by NMR that is not readily accessible from X-ray diffraction data.

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(18) For the present systems, the same corrections can be applied for scalar couplings derived from the generation of antiphase magnetization with respect to either H1′, H3′, or H5′/H5″ protons. The $T_{1,sel}$ (^{1}H) values are: Free DNA H1′, 240 ±20 ms; H3′, 200 ±10 ms; H5′/H5″, 180 ± 20 ms. Complex H1′, 120 ± 20 ms; H3′, 100 ± 20 ms; H5′/H5″, 110 ± 20 ms.

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(20) To estimate this correction, we consider a three-spin system consisting of proton A and the geminal protons K and L, where A and K, and A and L exhibit three-bond scalar couplings J_1 and J_2, respectively, with $J_1 > J_2$. *A*, *K*, and *L* denote the corresponding spin operators. The magnetization transfer $2A_xK_z \rightarrow 2A_xL_z$ mediated by K-L spin flips during 2δ is small compared with the generation of $2A_xK_z$ and $2A_xL_z$ coherence due to J_1 and J_2, and may thus be approximated by $d/dt[2A_xK_z(t)] = -\sigma_{KL}\{[2A_xK_z](t) - [2A_xL_z]\ (t)\} = -\sigma_{KL}$ (sin $\pi J_1 t$ − sin $\pi J_2 t$), where σ_{KL} is the cross-relaxation rate between K and L. Integration from t = 0 to t = 2δ with sin $\pi J_1 t \cong \pi J_1 t$ yields $\Delta[2A_xK_z] = -2\pi\delta^2\ \sigma_{KL}(J_1 - J_2)$, where $\Delta[2A_xK_z]$ represents the amount of antiphase magnetization transformed from $2A_xK_z$ into $2A_xL_z$ at $t = 2\delta$. The apparent scalar couplings, J_1^{app} and J_2^{app}, are then given by

$$J_i^{app} = (1/2\pi\delta)\arctan\{\sqrt{[(\sin\pi J_i 2\delta/\cos J_i 2\delta)^2[1 - \sigma_{KL}\delta(J_1 - J_2)/J_i]]}\}$$

with i = 1, 2. Setting J_1 and J_2 to the experimental values, the difference between experimental and calculated apparent coupling provides a good estimate for the correction.

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