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NMR Scalar Couplings Across Intermolecular Hydrogen Bonds Between Zinc-Finger Histidine Side Chains And DNA Phosphate Groups

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Abstract

NMR scalar couplings across hydrogen bonds represent direct evidence of the partial covalent nature of hydrogen bonds and provide structural and dynamic information on hydrogen bonding. In this article, we report heteronuclear ^{15}N - ^{31}P and ^1H - ^{31}P scalar couplings across the intermolecular hydrogen bonds between protein histidine (His) imidazole and DNA phosphate groups. These hydrogen-bond scalar couplings were observed for the Egr-1 zinc-finger–DNA complex. Although His side-chain NH protons are typically undetectable in heteronuclear ^1H - ^{15}N correlation spectra due to rapid hydrogen exchange, this complex exhibited two His side-chain NH signals around ^1H 14.3 ppm and ^{15}N 178 ppm at 35 °C. Through various heteronuclear multi-dimensional NMR experiments, these signals were assigned to two zinc-coordinating His side chains in contact with DNA phosphate groups. The data show that the $\text{N}_{\delta 1}$ atoms of these His side chains are protonated and exhibit the ^1H - ^{15}N cross peaks. Using heteronuclear ^1H , ^{15}N , and ^{31}P NMR experiments, we observed the hydrogen-bond scalar couplings between the His $^{15}\text{N}_{\delta 1}$ / $^1\text{H}_{\delta 1}$ and DNA phosphate ^{31}P nuclei. These results demonstrate the direct involvement of the zinc-coordinating His side chains in the recognition of DNA by the Cys₂His₂-class zinc fingers in solution.

Graphical abstract

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Introduction

Hydrogen bonds are extremely important for biological macromolecules to function. For example, intramolecular hydrogen bonds define the secondary structures of proteins and nucleic acids, and intermolecular hydrogen bonds play crucial roles in the molecular recognition and catalysis by these macromolecules. Hydrogen bonds are typically identified using the geometrical criteria of donor and acceptor groups in three-dimensional (3D) structures. Owing to progress in structural biology, numerous 3D structures of proteins, nucleic acids, and their complexes are available in the Protein Data Bank (PDB), providing a wealth of structural information on hydrogen bonds in biological systems.

However, any aspects beyond the geometrical features are only poorly understood for hydrogen bonds in biological macromolecules. The partially covalent nature of the hydrogen bonds had remained uncertain until it was experimentally verified by means of NMR scalar couplings across hydrogen bonds in the late 1990s (as reviewed by Grzesiek and co-workers).¹ These types of couplings (“hydrogen-bond scalar couplings”) were discovered initially for the hydrogen bonds of nucleic acid base pairs and protein secondary structures.²⁻⁵ Hydrogen-bond scalar couplings can be predicted from 3D structures by quantum chemical calculations as well as by empirical equations.¹ Hydrogen-bond scalar couplings provide direct evidence of hydrogen bonds and, in fact, are included in IUPAC’s recently updated criteria for hydrogen bonds.⁶ Hydrogen-bond scalar couplings can also provide information on hydrogen-bonding dynamics because these couplings are influenced by the transient distortion or breakage of the hydrogen bonds as well.⁷⁻¹⁰

To understand enzymatic catalysis and molecular recognition by proteins at an atomic level, characterizations of the intermolecular hydrogen bonds are vital. NMR scalar couplings across intermolecular hydrogen bonds have been observed for the molecular interfaces of nucleic acids (as reviewed in refs¹¹⁻¹²), protein–nucleic acid complexes,^{10,13-19} and other protein–ligand complexes²⁰⁻²¹. Some of the observations were made for side-chain moieties of proteins. For example, Löhr et al. observed hydrogen-bond scalar couplings between ¹H and ³¹P nuclei for serine (Ser) and threonine (Thr) side-chain OH groups interacting with riboflavin monophosphate (FMN) in a flavodoxin-FMN complex.²⁰ Liu et al. observed hydrogen-bond scalar couplings between ¹⁵N nuclei for the arginine (Arg) side chains interacting with RNA guanine bases.¹⁹ In our recent studies of several protein–DNA complexes, we observed hydrogen-bond scalar couplings between ¹⁵N and ³¹P nuclei for lysine (Lys) side-chain NH₃⁺ groups interacting with DNA phosphates.^{10,13-18}

In this paper, we demonstrate that the histidine (His) side chains also exhibit measurable heteronuclear ¹⁵N-³¹P and ¹H-³¹P scalar couplings across intermolecular hydrogen bonds with DNA phosphates. Usually, His side-chain NH protons are undetectable by NMR due to rapid hydrogen exchange with water.²² However, the His side-chain NH moieties interacting with DNA phosphates in the Egr-1 zinc-finger–DNA complex exhibited clearly observable heteronuclear ¹H-¹⁵N correlation signals. For these NH moieties, we have studied the hydrogen-bond scalar couplings between the His side-chain and DNA phosphate groups.

Experimental Methods

Protein and DNA

The ^{15}N - or $^{13}\text{C}/^{15}\text{N}$ -labeled Egr-1 zinc-finger protein (human Egr-1 residues 335–423) was expressed in *Escherichia coli* strain BL21 (DE3) and purified as previously described.^{23–25} In the following description, we adopt the residue-numbering scheme for Egr-1 (also known as Zif268) zinc fingers defined by Pabo and co-workers^{26–27} and used in many studies of Cys₂His₂ type zinc-finger proteins. In this scheme, the two key residues in the current paper, His25 and His53, correspond to His358 and His386 of the full-length human Egr-1 protein. Individual strands of 12-bp DNA containing the Egr-1 recognition sequence were purchased from Integrated DNA Technologies, Inc. and purified via Mono-Q anion-exchange chromatography as previously described.²⁴ The complementary strands were mixed and annealed, and the duplex was isolated using a Mono-Q anion exchange column.²⁴ NMR samples of ^{15}N - or $^{13}\text{C}/^{15}\text{N}$ -labeled Egr-1 zinc-finger–DNA complexes were prepared as previously described.¹⁶ The buffer conditions were 20 mM succinate•KOH (pH 5.8), 20 mM KCl, and 0.1 mM ZnCl₂. D₂O for NMR lock was separately sealed in a coaxial tube for each sample.¹⁶

NMR spectroscopy

All NMR experiments were performed at 35 °C using Bruker Avance III spectrometers operated at the ^1H -frequencies of 600, 750, or 800 MHz. The vast majority of protein backbone and side-chain ^1H , ^{13}C , and ^{15}N resonances of this complex were assigned in our previous work (Biological Magnetic Resonance Bank [BMRB] accession code: 26808).¹⁶ For observation of His imidazole signals, ^1H - ^{15}N HMQC²⁸ and long-range ^1H - ^{15}N HSQC²⁹ spectra were recorded for a 0.4 mM ^{15}N -labeled Egr-1–DNA complex sample. To observe NOE cross peaks for the His side-chain NH moieties, a two-dimensional (2D) ^1H - ^{15}N NOESY-HSQC spectrum²⁸ with the two ^1H dimensions was recorded with a ^{15}N carrier position at 175 ppm and a mixing time of 50 ms for the same sample. For assignment of His imidazole resonances, 3D ^1H - ^{13}C NOESY-HSQC and 2D (HB)CB(CGCD)HD³⁰ spectra were recorded for a 0.8 mM ^{13}C , ^{15}N -labeled Egr-1–DNA complex sample. All experiments involving ^{31}P pulses were conducted for the 0.4 mM ^{15}N -labeled complex sample using a cryogenic QCI $^1\text{H}/^{13}\text{C}/^{15}\text{N}/^{31}\text{P}$ probe operated at the ^1H -frequency of 600 MHz. The hydrogen-bond scalar coupling constants $^hJ_{\text{NP}}$ and $^hJ_{\text{HP}}$ were analyzed using the pulse sequences explained below. The NMR data were processed with the NMR-Pipe program³¹ and analyzed with the NMR-View program³².

Quantum chemical prediction of $^hJ_{\text{NP}}$ and $^hJ_{\text{HP}}$ couplings

Using the Density Functional Theory (DFT) method, $^hJ_{\text{NP}}$ and $^hJ_{\text{HP}}$ coupling constants for His25/His53 side chains and DNA phosphates were predicted from the crystal structure of the Egr-1–DNA complex (PDB 1AAY). These calculations were performed for a dimethylphosphate – methylimidazole complex using Gaussian 09 software³³. The coordinates of the DNA C_{3'}, O_{3'}, P, O_{1P}, O_{2P}, and O_{5'} atoms and the His C _{β} , C_T, N _{δ 1}, C _{δ 1}, C _{ϵ 1}, and N _{ϵ 2} atoms in the crystal structure were used for this system and the C_{3'}, C_{5'}, and C _{β} methylene groups were substituted to methyl groups. Based on experimental data, the calculations used the His tautomeric state with protonated N _{δ 1} and deprotonated N _{ϵ 2} atoms.

The overall charge of the system was set to -1 , which arises from the phosphate moiety. Before the DFT-based calculations of the coupling constants, the atomic coordinates (including those of the hydrogen atoms that are absent in the crystal structure) were optimized at the MP2/6-31+G(d,p) level³⁴, while constraining the positions of the C_β , $N_{\delta 1}$, $C_{\delta 1}$, P , C_3' , and C_5' atoms. Using the optimized structures, the $^hJ_{NP}$ and $^hJ_{HP}$ coupling constants were calculated at the B3LYP/6-311+G(2d,2p) level³⁵, as described previously.¹³

Results

1H - ^{15}N cross peaks at unusual chemical shifts

Figure 1 shows a 2D 1H - ^{15}N HMQC spectrum recorded for the complex of ^{15}N -labeled Egr-1 zinc-finger protein and unlabeled 12-bp DNA at 35 °C. This spectrum was measured with a relatively wide ^{15}N spectral width, covering a range from 66 to 184 ppm. The complex exhibited unusual 1H - ^{15}N signals around 1H 14.3 ppm and ^{15}N 178 ppm. The 1H chemical shifts of these signals are near a typical range of DNA imino 1H chemical shifts. However, these HMQC signals are not from DNA imino protons; first, the DNA duplex used is unlabeled; even if ^{15}N nuclei of natural abundance (0.4%) were detected, the observed ^{15}N chemical shifts are not consistent with the typical chemical shifts of thymine N3 (~159 ppm) or guanine N1 (~147 ppm) nuclei.

Signals arising from His side-chain $N_{\delta 1}$ - $H_{\delta 1}$ moieties

To assign these unique signals, we recorded long-range 1H - ^{15}N HSQC, 1H - ^{15}N NOESY-HSQC, 1H - ^{13}C NOESY-HSQC, and (HB)CB(CGCD)HD spectra for the Egr-1–DNA complex. Our previous 1H , ^{13}C , and ^{15}N resonance assignment data for the other moieties of the complex under the same experimental conditions facilitated this assignment process. Figure 2 shows some of the spectra used to assign these signals. A long-range HSQC spectrum for His side chains (Figure 2B) correlates the resonances of carbon-attached $^1H_{\delta 2}$ and $^1H_{\epsilon 1}$ nuclei with $^{15}N_{\delta 1}$ and $^{15}N_{\epsilon 2}$ resonances, mainly via two-bond scalar couplings, $^2J_{NH}$, and, to a far smaller degree, three-bond scalar couplings, $^3J_{NH}$.^{29,36} His $^1H_{\delta 2}$ resonances were assigned using 2D (HB)CB(CGCD)HD (Figure 2D) and 3D 1H - ^{13}C edited NOESY-HSQC spectra for aromatic residues. His $^1H_{\epsilon 1}$ resonances were assigned using the long-range 1H - ^{15}N HSQC (Figure 2B) and 3D 1H - ^{13}C NOESY-HSQC spectra. The long-range 1H - ^{15}N correlation spectrum also allows us to distinguish three possible tautomeric states of His side chains, as described by Pelton et al.³⁶ Our long-range 1H - ^{15}N HSQC spectrum clearly indicates that the His side chains observed in this spectrum are in the tautomeric state in which $N_{\delta 1}$ is protonated and $N_{\epsilon 2}$ is deprotonated.

Importantly, the long-range HSQC spectrum for the His side chains showed signals at the ^{15}N chemical shifts identical to those of the unique signals around 1H 14.3 ppm in the HMQC spectrum (see Figures 2A and 2B), indicating that these HMQC signals are from the His side-chain $NH_{\delta 1}$ moieties. His imidazole NH protons are typically undetectable by NMR spectroscopy due to rapid hydrogen exchange with water.²² The clearly detectable signals from these His side-chain $NH_{\delta 1}$ moieties suggest that their hydrogen-exchange rates are slowed due to hydrogen bonds and/or the lack of exposure to solvent.³⁷ The 1H - ^{15}N NOESY-HSQC spectrum for the His side-chain NH groups (Figure 2C) showed many NOE

cross peaks. Based on these NOEs and the ^{15}N chemical shifts, the two unique ^1H - ^{15}N cross peaks around ^1H 14.3 ppm and ^{15}N 178 ppm were unambiguously assigned to side-chain $\text{NH}_{\delta 1}$ moieties of His25 and His53, as shown in Figure 2A.

Zinc-coordinating His side chains that interact with DNA phosphate

The His25 and His53 side chains, which exhibited the unusual $^1\text{H}_{\delta 1}$ signals around 14.3 ppm, coordinate a zinc ion at N_{e2} and are in contact with DNA phosphate groups at $\text{N}_{\delta 1}$ in the 1.6 Å resolution crystal structure of the same complex (PDB 1AAY)²⁶ (Figure 3). Crystal structures at this resolution do not explicitly provide information about the protonation state of the His side chains. However, the distances between the $\text{N}_{\delta 1}$ and nearby phosphate oxygen atoms (2.79 Å for His25 and 2.89 Å for His53) in the crystal structure suggest that they form $\text{NH}_{\delta 1}\cdots\text{O}$ hydrogen bonds. In this sense, the crystal structure implicitly suggests that the $\text{N}_{\delta 1}$ atoms of these His side chains should be protonated. Our NMR data clearly show that these $\text{N}_{\delta 1}$ atoms are indeed protonated for these zinc-coordinating His side chains. Most likely, the intermolecular hydrogen bonds between the His $\text{NH}_{\delta 1}$ and DNA phosphate groups render hydrogen exchange far slower, allowing for the observation of the signals from the imidazole $\text{NH}_{\delta 1}$ moieties.

Hydrogen-bond scalar couplings between His side-chain and DNA phosphate nuclei

Using the pulse sequences shown in Figure 4, we examined two types of scalar couplings across the hydrogen bonds between the zinc-finger His side-chain and DNA phosphate groups. One type is three-bond coupling, $^hJ_{NP}$, between the His $^{15}\text{N}_{\delta 1}$ and phosphate ^{31}P nuclei. To analyze $^hJ_{NP}$ couplings, we conducted 2D H(N)P and 2D spin-echo $^hJ_{NP}$ -modulation difference constant-time HISQC experiments, as shown in Figure 4A and 4B, respectively. These experiments are analogous to what we previously designed for $^hJ_{NP}$ couplings between Lys side-chain NH_3^+ and DNA phosphate groups.^{13,40} Figure 5A shows the 2D H(N)P spectrum recorded for the ^{15}N -labeled Egr-1–DNA complex. The H(N)P experiment utilizes coherence transfers via $^hJ_{NP}$ couplings between His $^{15}\text{N}_{\delta 1}$ and DNA ^{31}P nuclei. Because of fast ^{15}N relaxation for His imidazole groups, we had to use relatively short periods for the coherence transfers, compared to those in the corresponding experiments for the Lys side-chain NH_3^+ groups. Despite the use of the relatively short time for coherence transfers (31 ms for each), the H(N)P spectrum clearly showed signals from His25 and His53 $^1\text{H}_{\delta 1}$ nuclei at the ^{31}P chemical shifts of –5.5 ppm and –6.8 ppm, respectively, suggesting that $^hJ_{NP}$ couplings are sizable for these His side chains. In fact, the absolute values of the $^hJ_{NP}$ coupling constants measured by $^hJ_{NP}$ -modulation difference constant-time HISQC experiment were as large as 2 Hz, as shown in Table 1. Interestingly, these couplings are substantially larger than $^hJ_{NP}$ couplings those between Lys side-chain NH_3^+ and DNA phosphate groups.^{10,13–18} Although the ^{31}P chemical shifts observed in the H(N)P spectrum (Figure 5A) are somewhat unusual for canonical B-form DNA (typically ranging from –3 to –5 ppm),⁴¹ a one-dimensional (1D) ^{31}P spectrum recorded for the same sample clearly shows signals at the same ^{31}P chemical shifts (indicated by arrows in Figure 5C). These unique ^{31}P chemical shifts are likely caused by the strong interactions with the His side chains.

Comparison with quantum chemical calculations

Using the DFT method, we predicted $^hJ_{NP}$ and $^hJ_{HP}$ coupling constants from the crystal structure of the Egr-1 – DNA complex. The magnitudes of the $^hJ_{NP}$ couplings for His25 and His53 were predicted to be 0.47 Hz and 0.69 Hz, respectively. Those of the $^hJ_{HP}$ coupling constants for His25 and His53 were predicted to be 0.42 Hz and 1.01 Hz, respectively. Although both the DFT-based prediction and the NMR data show significant magnitudes of the $^hJ_{NP}$ and $^hJ_{HP}$ couplings across the hydrogen bonds between the His side chains and DNA phosphate groups, the computational and experiment data agree only qualitatively. This might be due to the dynamic nature of the intermolecular interactions. In their computational work, Czernek and Brüschweiler showed that $^hJ_{NP}$ and $^hJ_{HP}$ coupling constants for phosphate groups are sensitive to slight difference in hydrogen-bonding geometry.⁴³ Due to this, conformational fluctuations of the His side chains and the DNA phosphate groups may significantly affect these couplings.

Discussion

Our current NMR data unequivocally demonstrate that the zinc-coordinating side chains His25 and His53 of the Egr-1 zinc-finger protein form intermolecular hydrogen bonds with DNA phosphate groups in solution. Egr-1 (Zif268) belongs to the Cys₂-His₂-type zinc-finger protein family, the most common family of eukaryotic transcription factors, and has been studied as an archetype of this family and as a scaffold for zinc-finger technology.^{44–46} Because the zinc-coordinating His residues are conserved, it is likely that some other natural and artificial Cys₂-His₂ zinc-finger proteins can also form the same type of intermolecular hydrogen bonds with DNA phosphate groups. However, this is not always the case. For example, in our current work, only the His25 and His53 side chains in the first and second zinc fingers of Egr-1 exhibited this kind of hydrogen bond, whereas the corresponding side chain, His81, in the third zinc finger did not. This is also consistent with the crystal structure, which shows no intermolecular hydrogen bond for His81.

As demonstrated above, hydrogen-bond scalar couplings and unique ¹H chemical shifts present signatures for the hydrogen bonds of His side chains. It should be noted that if the residence time, τ , of the protein bound to DNA is relatively short and satisfies $\tau < |2\pi J|^{-1}$, observations of the intermolecular hydrogen-bond scalar couplings would be difficult due to self-decoupling.¹⁰ Because the residence time is ~ 1000 s for the Egr-1 zinc-finger–DNA complex,²⁴ the self-decoupling effect is negligible for the current system. We expect that quantitative investigations of ¹⁵N relaxation and hydrogen exchange will provide further insight into the intermolecular hydrogen bonds between the His imidazole and DNA phosphate groups.

Conclusions

Using NMR spectroscopy, we have demonstrated evidence of the intermolecular hydrogen bonds between His side-chain and DNA phosphate groups in the Egr-1–DNA complex in solution. Although His imidazole NH moieties are typically undetectable due to rapid hydrogen exchange, those forming the intermolecular hydrogen bonds with DNA were clearly observed in heteronuclear ¹H-¹⁵N correlation experiments, presumably due to slower

hydrogen exchange. The heteronuclear ^1H - ^{31}P and ^{15}N - ^{31}P scalar couplings across the hydrogen bonds were found to be as large as 2 Hz. This is far larger than those previously observed for Lys side-chain NH_3^+ and DNA phosphate groups of several protein-DNA complexes. Further NMR studies of the intermolecular hydrogen bonds between proteins and DNA will help us understand DNA recognition by proteins.

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Figure 1. ^1H - ^{15}N HMQC spectrum recorded at 35 °C for the ^{15}N -labeled Egr-1 zinc-finger protein–DNA complex (0.4 mM) in a buffer of 20 mM potassium succinate (pH 5.8), 20 mM KCl, and 0.1 mM ZnCl_2 . The 12-bp DNA was unlabeled. The two signals from the interfacial His side-chain $\text{NH}_{\delta 1}$ moieties are indicated by a green arrow.

Figure 2.

Assignment of the signals from the His side-chain $\text{NH}_{\delta 1}$ moieties of the Egr-1 zinc-finger-DNA complex. (A) ^1H - ^{15}N HMQC signals around ^1H 14.3 ppm and ^{15}N 178 ppm. (B) Long-range ^1H - ^{15}N correlation HSQC spectrum. (C) 2D ^1H - ^{15}N NOESY-HSQC spectrum for the His $\text{NH}_{\delta 1}$ moieties. (D) His region in the (HB)CB(CGCD)HD spectrum³⁰ for aromatic side chains.

Figure 3.

Two zinc-coordinating His side chains that make contact with DNA phosphate groups in the crystal structure of the Egr-1 (Zif268) zinc-finger protein–DNA complex (PDB code: 1AAY). The shortest distances between the His $N_{\delta 1}$ and phosphate oxygen atoms are 2.79 Å and 2.89 Å for His25 and His53, respectively (indicated by dotted lines).

Figure 4.

Pulse sequences for observations of heteronuclear ^{15}N - ^{31}P and ^1H - ^{31}P scalar couplings h^3J_{NP} and h^2J_{HP} across the intermolecular hydrogen bonds between His side-chain and DNA phosphate groups. Thin and bold bars represent hard rectangular 90° and 180° pulses, respectively. Short-bold bars represent water-selective soft-rectangular ^1H 90° pulses (1.2 ms). Shaped pulses are ^1H half-Gaussian 90° pulses (2.1 ms) and ^{15}N r-SNOB³⁸ 180° pulses (1.0 ms). Unless indicated otherwise, pulse phases are along x . Pulsed field gradients were optimized to minimize the water signal. The acquisition time for the t_2 time domain (^1H dimension) was 54 ms. Quadrature detection in the t_1 domain was achieved using States-TPPI³⁹ for ϕ_1 . Based on the magnitudes of the $^1J_{NH}$ coupling constants for the His imidazole NH moieties (~ 98 Hz from F_1 - ^1H -coupled ^1H - ^{15}N HSQC), the delays common to these pulse sequences were as follows: $\tau = 2.3$ ms; $\tau_d = \tau - 1.2$ ms; and $\delta = 5.1$ ms. Carrier positions: ^1H , the position of the water resonance; ^{15}N , 175 ppm; and ^{31}P , -3.6 ppm (referenced to trimethylphosphate). Decoupling rf strengths: ^1H WALTZ, 3.47 kHz; ^{15}N GARP, 1.25 kHz; and ^{31}P WALTZ, 1.0 kHz. (A) 2D H(N)P experiment for heteronuclear ^1H - ^{31}P correlation between His side-chain NH and DNA phosphate resonances via coherence transfers through h^3J_{NP} couplings. Phase cycles were $\phi_1 = [2x, 2(-x)]$; $\phi_2 = [4x, 4(-x)]$; $\phi_3 = [x, -x]$; $\phi_4 = [8x, 8y, 8(-x), 8(-y)]$; and receiver = $[x, -x, -x, x, 2(-x, x, x, -x), x, -x, -x, x]$. $T_A = 31$ ms was used for ^{15}N - ^{31}P coherence transfers. The acquisition time for the t_1 domain (^{31}P dimension) was 16 ms. (B) 2D spin-echo h^3J_{NP} -modulation difference constant-time ^1H - ^{15}N HSQC experiment for quantitative measurements of h^3J_{NP} coupling constants. Phase cycles were $\phi_1 = [2x, 2(-x)]$; $\phi_2 = [4x, 4(-x)]$; $\phi_3 = [y, -y]$; $\phi_4 = [8x, 8y, 8(-x), 8(-y)]$; and receiver = $[x, -x, -x, x, 2(-x, x, x, -x), x, -x, -x, x]$. $T_B = T_A - \delta$ was used for h^3J_{NP} modulation. Two datasets were recorded in an interleaved manner with the two ^{31}P 180° pulses located at either positions a or b. For the position a, the signal intensity is modulated by evolution of h^3J_{NP} couplings, whereas for the position b, this modulation is cancelled. (C) 2D long-range ^1H - ^{31}P HMQC experiment for the correlation between His imidazole NH and DNA phosphate resonances via coherence transfers through h^2J_{HP} couplings. Phase cycles were $\phi_1 = [x, -x]$; $\phi_2 = [4x, 4(-x)]$; $\phi_3 = [2(x, 2(-x)]$; and receiver = $[x, -x, -x, x, -x, x, x, -x]$. $T_C = 22$ ms was used for ^1H - ^{31}P coherence transfers via h^2J_{HP} couplings. The acquisition time for the t_1 domain (^{31}P dimension) was 16 ms. (D) 2D spin-echo h^2J_{HP} -modulation difference ^1H - ^{15}N HSQC experiment for quantitative measurements of h^2J_{HP} coupling constants. Phase cycles are $\phi_1 = [x, -x]$; $\phi_2 = [2x, 2(-x)]$; $\phi_3 = [4y, 4(-y)]$; and receiver = $[x, -x, -x, x, -x, x, x, -x]$. $T_D = 17.9$ ms $[= 7/(4^1J_{NH})]$ was used for evolutions of h^2J_{HP} and $^1J_{NH}$ couplings. Two datasets were recorded in an interleaved

manner with the phase ψ being either x or $-x$. With $\psi = x$, signal intensity is modulated by $h^2 J_{HP}$ evolution during the first INEPT scheme, whereas with $\psi = -x$, this modulation is cancelled.

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Figure 5.

Observation of NMR signals arising from scalar couplings across the intermolecular hydrogen bonds between His imidazole and DNA phosphate groups for 0.4 mM ^{15}N -labeled Egr-1 zinc-finger–DNA complex. These spectra show cross peaks of His $^1\text{H}_{\delta 1}$ and DNA ^{31}P resonances. (A) H(N)P spectrum recorded with 512 scans per free induction decay (FID) using the pulse sequence shown in Figure 4A. (B) Long-range ^1H - ^{31}P HMQC spectrum recorded with 2112 scans per FID using the pulse sequence shown in Figure 4C. (C) 1D ^{31}P spectrum measured for all ^{31}P nuclei in the same sample. Arrows indicate ^{31}P resonances that were also observed in the spectra of the panels A and B. The ^{31}P chemical shifts are referenced to trimethylphosphate.

Through 2D long-range ^1H - ^{31}P HMQC (Figure 4C) and spin-echo $^hJ_{HP}$ -modulation difference HSQC (Figure 4D) experiments, we also investigated the two-bond couplings, $^hJ_{HP}$, between the His imidazole $^1\text{H}_{\delta 1}$ and phosphate ^{31}P nuclei across the hydrogen bonds. Because ^1H relaxation is faster than ^{15}N relaxation, these experiments required shorter times for the $^hJ_{HP}$ evolution than those for the $^hJ_{NP}$ evolution in the other two experiments. Figure 5B shows the 2D long-range ^1H - ^{31}P HMQC spectrum recorded with ^{15}N decoupling for the t_2 domain. This spectrum also exhibited ^1H - ^{31}P cross peaks at the same locations as those in the H(N)P spectrum. This indicates that the $^hJ_{HP}$ couplings between the His $^1\text{H}_{\delta 1}$ and DNA ^{31}P nuclei are also sizable. The $^hJ_{HP}$ coupling constants measured by the spin-echo $^hJ_{HP}$ -modulation difference HSQC experiment are given in Table 1. The absolute values of these coupling constants were also as large as 2 Hz. These $^hJ_{NP}$ and $^hJ_{HP}$ data represent direct evidence of the intermolecular hydrogen bonds between the zinc-finger His side-chain and DNA phosphate groups.

Table 1

Magnitudes of hydrogen-bond scalar couplings across the hydrogen bonds between the His side-chain and DNA phosphate groups measured for 0.4 mM ^{15}N -labeled Egr-1 zinc-finger–DNA complex. ^{a)}

	$ ^hJ_{NP} $ (Hz) ^{b)}	$ ^hJ_{HP} $ (Hz) ^{a)}
His25	2.1 ± 0.1	2.1 ± 0.4
His53	2.4 ± 0.1	2.2 ± 0.3

^{a)} The absolute values were calculated by $|^hJ| = \cos^{-1}(I_b / I_a) / (\pi T)$,^{40,42} where I_b and I_a are the signal intensities in the sub-spectra recorded with and without hJ modulation and T is the total length of the hJ -modulation period (i.e., 62 ms for $^hJ_{NP}$ and 35.8 ms for $^hJ_{HP}$). The uncertainties were estimated from the noise standard deviations of the sub-spectra.

^{b)} Measured with 192 scans per FID using the pulse sequence shown in Figure 4B.

^{c)} Measured with 192 scans per FID using the pulse sequence shown in Figure 4D.