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The Mobility of Histidine Side Chains Analyzed with ^{15}N NMR Relaxation and Cross-Correlation Data: Insight into Zinc-Finger – DNA Interactions

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Abstract

Due to chemical exchange, the mobility of histidine (His) side chains of proteins is typically difficult to analyze by NMR spectroscopy. Using an NMR approach that is uninfluenced by chemical exchange, we investigated internal motions of the His imidazole NH groups that directly interact with DNA phosphates in the Egr-1 zinc-finger – DNA complex. In this approach, the transverse and longitudinal cross-correlation rates for ^{15}N chemical shift anisotropy (CSA) and ^{15}N - ^1H dipole-dipole relaxation interference were analyzed together with ^{15}N longitudinal relaxation rates and heteronuclear Overhauser effect data at two magnetic field strengths. We found that the zinc-coordinating His side chains directly interacting with DNA phosphates are strongly restricted in mobility. This makes a contrast to the arginine and lysine side chains that retain high mobility despite their interactions with DNA phosphates in the same complex. The entropic effects of side-chain mobility on molecular association are discussed.

Graphical Abstract

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Supporting Information: The following file is available free of charge:
Experimental details (PDF)

The authors declare no competing financial interests.

Molecular recognition by proteins involves polar and charged side chains that form intermolecular hydrogen bonds and/or ion pairs with target molecules.¹ While these hydrogen bonds and ion pairs produce an enthalpic advantage, they may also cause an entropic disadvantage through restriction of internal motions of the side chains. Recently, we found that some lysine (Lys) and arginine (Arg) side chains interacting with DNA phosphates are highly mobile due to dynamic equilibria between the contact ion-pair (CIP) state and the solvent-separated ion-pair (SIP) state.^{2–6} This high mobility of intermolecular ion pairs should reduce entropic loss upon molecular association. In contrast, Arg side chains that interact with DNA bases were found to become rigid upon binding.^{3–4} These results clearly show a diversity in the entropic cost for individual side chains in molecular association events.

To gain further information on basic side chains, our current NMR study focuses on the mobility of interfacial histidine (His) side chains of the Egr-1 (Zif268) zinc-finger – DNA complex. ¹⁵N relaxation analysis of His side chains is practically difficult due to chemical exchange involving rapid hydrogen exchange with water⁷ as well as dynamic transitions between distinct protonation states.⁸ In favorable cases, however, hydrogen bonding may hinder the hydrogen exchange of some His side chains, allowing NMR detection of their labile ¹H nuclei. This is indeed the case for His25 and His53 of the Egr-1 zinc fingers in the specific complex with target DNA (Figure 1A).⁹ In heteronuclear ¹H-¹⁵N correlation spectra, signals from the imidazole N_δ1H groups of these His side chains are observed at approximately ¹⁵N 178 ppm and ¹H 14.2 ppm at 25°C (Figure 1B). The crystal structure¹⁰ show that His25 and His53 coordinate zinc ions at the N_ε2 atoms and form hydrogen bonds with DNA phosphates at the N_δ1 atoms (Figure 1A). Our previous NMR study showed that the ¹⁵N_δ1 and ¹H_δ1 nuclei of these His side chains exhibit sizable hydrogen-bond scalar

couplings to DNA ^{31}P nuclei.⁹ In the current study, we investigate the mobility of these interfacial His side chains in terms of the generalized order parameters (S^2).

In general, His side chains undergo chemical exchange between distinct protonation states,⁸ which can increase apparent NMR transverse relaxation rates.^{11–12} Presumably due to such an effect in the fast exchange regime, data quality in most NMR experiments for the His25 and His53 imidazole $\text{N}_{\delta 1}\text{H}$ groups was worse at the ^1H frequency of 800 MHz than that at 600 MHz. The presence of chemical exchange makes it difficult to determine the order parameters for the His side-chain $\text{N}_{\delta 1}\text{H}$ groups through conventional ^{15}N relaxation methods even though hydrogen exchange is slow enough to detect ^1H signals from these groups. To overcome this problem, we took advantage of sizable interference between ^{15}N chemical shift anisotropy (CSA) and ^{15}N - ^1H dipole-dipole (DD) relaxation mechanisms (i.e., CSA-DD cross-correlation) for the His imidazole $\text{N}_{\delta 1}\text{H}$ groups. The significant magnitude of $^{15}\text{N}_{\delta 1}$ CSA-DD cross-correlation for these side-chain groups is even qualitatively obvious from the F_2 - ^1H -coupled HSQC spectrum (Figure 1B), in which individual components of doublets due to a one-bond ^{15}N - ^1H scalar coupling ($|^1J_{\text{NH}}| = 98 \text{ Hz}$) significantly differ in ^{15}N line shape.^{13–14} By analyzing CSA-DD cross-correlation rates quantitatively, information on mobility can be obtained without adverse influences of chemical exchange.¹⁵ This feature is practically useful for analyzing the mobility of His side chains.

To investigate the dynamic properties of the His imidazole $\text{N}_{\delta 1}\text{H}$ groups, we measured the CSA-DD cross-correlation rates for ^{15}N transverse and longitudinal magnetizations at ^1H frequencies of 800 and 600 MHz. The pulse sequences for these measurements, which were originally developed for backbone NH groups by Bax, Tjandra, Palmer, and their co-workers,^{16–17} were optimized for His imidazole $\text{N}_{\delta 1}\text{H}$ group to account for differences in $^1J_{\text{NH}}$ coupling, ^{15}N chemical shifts, and spin systems. In these measurements, the intensities of signals (I_{cross}) arising from CSA-DD cross correlation were compared to those of corresponding control signals that underwent auto-relaxation (I_{auto}). The ratio of these intensities $I_{\text{cross}}/I_{\text{auto}}$ is given by $\tanh(\eta\tau)$, where η is the cross-correlation rate and τ is the total length of the period for cross-correlation.^{16–17} Figures 2A and 2B show data obtained in the transverse and longitudinal cross-correlation experiments for the His imidazole $\text{N}_{\delta 1}\text{H}$ groups. The transverse cross-correlation rates η_{xy} and the longitudinal cross-correlation rates η_z measured for the His imidazole $\text{N}_{\delta 1}\text{H}$ groups are shown in Table I. The η_{xy} rates measured for the His imidazole $\text{N}_{\delta 1}\text{H}$ groups were as large as $12\text{--}16 \text{ s}^{-1}$. These results alone implicate that these His $\text{N}_{\delta 1}\text{H}$ groups are not highly mobile.

To determine the order parameters S^2 for the His side-chain $\text{N}_{\delta 1}\text{H}$ groups, we conducted fitting calculations using the CSA-DD correlation rates η_{xy} and η_z together with ^{15}N longitudinal relaxation rates (R_1) and heteronuclear ^1H - ^{15}N nuclear Overhauser effect (NOE) data (Figure 2 and Table I). Importantly, since η_{xy} rates are used instead of R_2 rates, this method for determination of the order parameters is not impacted by chemical or conformational exchange with minor states (e.g., a deprotonated state) of the His imidazole $\text{N}_{\delta 1}\text{H}$ groups. For each His side chain, the fitting calculations utilized 8 data of 4 distinct relaxation parameters (i.e., η_{xy} , η_z , R_1 , and NOE) at 2 different magnetic field strengths together with the rotational diffusion parameters determined from backbone ^{15}N relaxation data for the same system³. Based on a neutron diffraction study,¹⁸ the distance between the

His side-chain $^{15}\text{N}_{\delta 1}$ and $^1\text{H}_{\delta 1}$ nuclei was assumed to be 1.07 Å. The reduced spectral density function for an axially symmetric diffusion model (Eq. 6 in Tjandra et al.¹⁹) was used to calculate the cross-correlation rates and other relaxation parameters, as described in Supporting Information. The fitting calculations optimized 4 fitting parameters: the order parameter S^2 , the internal motion correlation time τ_i , the ^{15}N CSA σ , and the angle θ between the NH bond vector and the main principal axis of ^{15}N CSA tensor. Since the ^{15}N CSA parameters were unknown for zinc-coordinating His side chains, the parameters σ and θ were treated as fitting parameters in these calculations.

The determined values of the parameters S^2 , τ_i , σ , and θ are listed in Table I. Uncertainties estimated by the Monte Carlo method show that the order parameter S^2 is best defined among the 4 fitting parameters. When the $\text{N}_{\delta 1}\text{H}$ bond length was changed from 1.07 Å to 1.05 Å or 1.09 Å, each fitting calculation from the current dataset gave only a ~1% difference from the original value of the order parameter S^2 . We found the $^{15}\text{N}_{\delta 1}$ CSA σ to be ~-210 ppm for the zinc-coordinating His side chains. This is slightly (10–15%) larger than the value determined for a non-metal coordinating histidine $\text{N}_{\delta 1}\text{H}$ group by a solid-state NMR.²⁰ However, the difference in $^{15}\text{N}_{\delta 1}$ CSA between the His imidazole moieties with and without a $\text{N}_{\epsilon 2}\text{-Zn}$ bond is not surprising because the solid-state NMR study also showed a significant difference in $^{15}\text{N}_{\delta 1}$ CSA between imidazole moieties with and without a $\text{N}_{\epsilon 2}\text{-H}$ bond.²⁰

The order parameters (S^2) determined for the His25 and His53 imidazole $\text{N}_{\delta 1}\text{H}$ groups indicate that their internal motions are strongly restricted in the Egr-1–DNA complex. The S^2 values for these His imidazole $\text{N}_{\delta 1}\text{H}$ groups are larger than typical order parameters ($S^2 \sim 0.85$) for backbone NH groups in secondary structures of proteins. The motion restriction of these His imidazole groups are probably due to simultaneous interactions with zinc and DNA phosphate (Figure 1A). It should be noted that some Arg and Lys side chains are highly mobile even when they interact with DNA phosphates forming intermolecular ion pairs in protein-DNA complexes.^{2–6,22–23} Figure 3 compares the order parameters of the His imidazole side chains (this work) to those of the Arg and Lys side chains (our previous work)³ that interact with DNA phosphates in the same Egr-1 zinc-finger–DNA complex. For example, the Lys79 NH_3^+ group exhibited an order parameter for its symmetry axis (S^2_{axis}) of 0.26, although scalar $^{15}\text{N}\text{-}^{31}\text{P}$ coupling across the intermolecular hydrogen bond to DNA phosphate indicates a direct interaction between the Lys side chain and DNA phosphate.²² Such differences in mobility are qualitatively consistent with the crystallographic B -factors of the His25 $\text{N}_{\delta 1}$ (23 Å²), His53 $\text{N}_{\delta 1}$ (17 Å²), and Lys79 N_{ζ} (33 Å²) atoms in the 1.6-Å resolution X-ray structure of the same complex (PDB 1AAY). By retaining high mobility, the Arg and Lys side chains may reduce the entropic cost for binding.

Although the His25 and His53 side chains are rigid in the complex, this rigidity would not cause entropic loss upon the protein's binding to DNA if these side chains were rigid in the free state as well. Unfortunately, examination of this possibility by the same NMR approach was not possible due to rapid hydrogen exchange in the free state. As an alternative approach, we analyzed 0.6-μs molecular dynamics (MD) trajectories from our previous studies^{3,22} for the Egr-1 zinc-finger protein and its complex with 12-bp target DNA. As shown in Supporting Information, the MD-derived auto-correlation functions for the

imidazole N_{δ1}H bond vectors for His25 and His53 in the free and DNA-bound states MD simulations showed that these His side chains are rigid in both states. The MD-derived S^2 parameters in the DNA-bound state were 0.87 and 0.88 for the His25 and His53 side chains, respectively, whereas those in the free state were 0.87 and 0.82 for His25 and His53, respectively. Thus, it seems that these zinc-coordinating His side chains can enjoy an enthalpic gain from the formation of intermolecular hydrogen bonds without the expense of an entropic loss arising from a decrease in mobility. In medicinal chemistry, it is well known that conformational rigidification of drugs can lead to affinity enhancement due to entropic effects.^{24–25} Likewise, for a high-affinity binding to DNA, zinc fingers may take advantage of the rigidity of the zinc-coordinating His side chains in the free state.

In conclusion, our investigations using NMR relaxation and cross-correlation data have provided information on the internal motions of the zinc-coordinating His side chains of the Egr-1 zinc fingers at the interface with DNA. Although His side chains typically undergo chemical exchange between different protonation sites, such exchange processes do not influence our analysis because it does not utilize transverse relaxation data. The results from our experiments unequivocally indicate that the internal motions of these zinc-coordinating His side chains are highly restricted. This makes clear contrast to some Arg and Lys side chains that interact with DNA phosphates but retain relatively high mobility in the same protein-DNA complex.

EXPERIMENTAL SECTION

All NMR experiments were performed for a 0.4 mM solution of the Egr-1 zinc-finger (¹⁵N) – DNA complex at 25°C using Bruker Avance III spectrometers equipped with cryogenic probes at ¹H frequencies of 800 and 600 MHz. As previously described,³ a 370-μl solution of 0.4 mM complex in a buffer of 20 mM d₄-succinate•KOH (pH 5.8), 20 mM KCl, and 0.1 mM ZnCl₂ was sealed in a Norell 5-mm NMR co-axial tube in which an inner stem tube containing D₂O was inserted. The separation of D₂O avoids any adverse effects due to the exchange between N_{δ1}H and N_{δ1}D, for which ¹⁵N chemical shifts are different. The ¹H and ¹⁵N resonances of the His side chains were previously assigned.⁹ The fitting calculations to determine the order parameters and CSA parameters were conducted using a Mathematica script. Other experimental and computational details are described in the Supporting Information.

Supplementary Material

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Figure 1.

The His25 and His53 side chains at the molecular interface of the complex of the Egr1 zinc-fingers with its target DNA. **(A)** Positioning of His25 and His53 in a crystal structure (PDB 1AAY). These His side chains directly interact with zinc (shown in purple) and DNA phosphate (red and orange). **(B)** Signals from the His25 and His53 imidazole $\text{N}_{\delta 1}\text{H}$ groups observed in a ^1H - ^{15}N HSQC spectrum recorded for the Egr-1–DNA complex at 25°C. **(C)** An F_1 - ^1H -coupled HSQC spectrum recorded for the same sample. An F_1 slice at the position indicated by an arrow is also shown.

Figure 2.

^{15}N relaxation and cross-correlation measurements of for the imidazole $\text{N}_{\delta 1}\text{H}$ groups of His25 (blue) and His53 (red) at the ^1H frequency of 600 MHz. (**A**, **B**) Data for measurements of the ^{15}N CSA / ^{15}N - ^1H DD cross-correlation rates η_{xy} (Panel A) and η_z (Panel B). Solid lines show the best-fit curves obtained with $I_{cross}/I_{auto} = \tanh(\eta\tau)$.¹⁵ (**C**) Data for measurements of the ^{15}N longitudinal relaxation rates R_1 . (**D**) F_1 slices of the spectra recorded with (black) and without (magenta) ^1H saturation in the heteronuclear [^1H]- ^{15}N NOE experiment.

Figure 3.

Order parameters S^2 for the side-chain moieties that form a direct hydrogen bond to a DNA phosphate in at least one of the crystal structures of the wild-type Egr-1 zinc-finger – unmodified DNA complexes. **(A)** Order parameters for His imidazole N δ 1H bonds from the current study. **(B)** Order parameters of the Arg N ϵ H and Lys NH $_3^+$ groups from our previous NMR study.³

Table 1.

Order parameters and other fitting parameters determined from the ^{15}N relaxation data for His side-chain $\text{N}_{\delta 1}\text{H}$ groups.

	His25	His53
Relaxation data at 800 MHz ^{a)}		
$^{15}\text{N } R_1 [\text{s}^{-1}]$	$0.76 \pm 0.04 (0.79)$	$0.74 \pm 0.05 (0.77)$
$[\text{H}]\text{N NOE}$	$0.79 \pm 0.06 (0.81)$	$0.85 \pm 0.06 (0.85)$
$\eta_{xy} [\text{s}^{-1}]$	$15.8 \pm 1.2 (16.5)$	$15.6 \pm 1.1 (16.2)$
$\eta_z [\text{s}^{-1}]$	$0.50 \pm 0.10 (0.50)$	$0.45 \pm 0.09 (0.48)$
Relaxation data at 600 MHz ^{a)}		
$^{15}\text{N } R_1 [\text{s}^{-1}]$	$1.03 \pm 0.02 (0.97)$	$1.05 \pm 0.03 (1.00)$
$[\text{H}]\text{N NOE}$	$0.76 \pm 0.04 (0.73)$	$0.82 \pm 0.04 (0.82)$
$\eta_{xy} [\text{s}^{-1}]$	$12.6 \pm 0.5 (12.5)$	$12.1 \pm 0.6 (12.3)$
$\eta_z [\text{s}^{-1}]$	$0.62 \pm 0.07 (0.60)$	$0.73 \pm 0.04 (0.62)$
Fitting parameters ^{b)}		
S^2	0.91 ± 0.04	0.98 ± 0.07
$\tau_i [\text{ns}]$	1.0 ± 0.3	0.3 ± 0.4
$\sigma [\text{ppm}]$	-207 ± 22	-213 ± 22
$\theta [^\circ]$	29 ± 4	29 ± 2

^{a)} Values in parentheses are those from the fitting calculations. η_{xy} , the CSA-DD cross-correlation rate; η_z , CSA-DD cross-correlation rate.

^{b)} S^2 , the generalized order parameter; τ_i the correlation time for internal motion; σ , the effective ^{15}N CSA; θ , the angle between the ^{15}N CSA main principal axis and the $\text{N}_{\delta 1}\text{H}$ bond. The molecular rotational correlation time $\tau_r = 13.9 \text{ ns}$ and the rotational anisotropy ($D_{||}/D_{\perp}$) of 1.59 from the previous study³ were used for the fitting calculations. The Monte Carlo method²¹ was used to estimate uncertainties.